NATURE

No. 4808 SATURDAY, DECEMBER 23, 1961 Vol. 192

CONTENTS

	Page
TECHNICAL AND SCIENTIFIC MAN-POWER IN BRITAIN	1105
CAPTAIN COOK'S VOYAGES. By Prof. R. Firth	1107
FLORA OF NEW ZEALAND. By Dr. George Taylor	1108
SAMPLING AND ANALYSIS OF COMBUSTION PRODUCTS. By Dr. C. F. Cullis	1108
THE ENZYMES. By Dr. Edwin C. Webb	1109
IDENTIFICATION, PROPERTIES AND USES OF INDIAN TIMBERS. By Dr. C. R. Metcalfe	1110
THE BETTER USE OF THE WORLD'S FAUNA FOR FOOD. By Dr. H. D. Kay, C.B.E., F.R.S.	1112
FIFTH INTERNATIONAL CONGRESS OF BIOCHEMISTRY. By Prof. W. T. J. Morgan, C.B.E., F.R.S.	1115
ATTENTION AND IN-ATTENTION IN NEUROPHYSIOLOGY. By L. Goldie and Mrs. J. M. Green	1116
AMINO-ACID SEQUENCE OF HORSE HEART CYTOCHROME *C*. By Dr. E. Margoliash and Prof. Emil L. Smith; Dr. G. Kreil and Prof. H. Tuppy; Dr. E. Margoliash, Prof. Emil L. Smith, Dr. Gunther Kreil and Prof. Hans Tuppy	1121
OBITUARIES:	
PROF. M. W. TRAVERS, F.R.S. By Prof. D. H. Everett	1127
PROF. R. H. DASTUR, O.B.E. By Prof. R. D. Asano	1128
NEWS AND VIEWS	1129
THE NEW MATHEMATICS AND OCEANOGRAPHY BUILDING OF THE UNIVERSITY OF LIVERPOOL. By Prof. L. Rosenhead, C.B.E., F.R.S.	1133
OXIDATION PROCESSES IN CHEMICAL MANUFACTURE. By Prof. F. Morton	1134
FAST REACTIONS IN PHYSICAL CHEMISTRY. By Dr. W. Gerrard	1136
METALLURGY OF BERYLLIUM. By Prof. N. J. Petch	1137
CHANGES OF CLIMATE. By Dr. R. C. Sutcliffe, O.B.E., F.R.S.	1139
CHEMICAL WARFARE ON PLANT PESTS AND DISEASES. By W. C. Moore, C.B.E.	1140
THE SOVIET HYDROMETEOROLOGICAL SERVICE. By R. F. Zobel	1141
POPULATION TRENDS IN THE U.S.S.R. AND THE UNITED STATES	1142
NEW SYNTHETIC DETERGENTS. By Dr. Nora H. Johnson	1142
PRODUCTIVITY IN LETTERPRESS PRINTING	1143
A SPECTROSCOPIC SURVEY OF ENGLISH AND CONTINENTAL MEDIEVAL GLAZED POTTERY. By L. C. Thomas and J. W. G. Musty	1143
CÆSIUM-137 AND STRONTIUM-90 IN PRECIPITATION, SOIL AND ANIMALS IN NORWAY. By T. Hvinden and A. Lillegraven	1144
A WORKING MODEL FOR THE UPPER ATMOSPHERE. By Luigi G. Jacchia	1147
OPTICAL CALIBRATION OF SOME MONODISPERSE POLYSTYRENE LATEXES IN ARRAYS. By Dr. Herbert E. Kubitschek	1148
STRUCTURES AND CONFORMATIONS OF SOME NATURALLY OCCURRING DERIVATIVES OF PROLINE. By R. J. Abraham, K. A. McLauchlan, Shirley Dalby, Prof. G. W. Kenner, R. C. Sheppard and L. F. Burroughs	1150
RELATIONSHIP BETWEEN OXIDATION OF ISOCITRATE AND METABOLISM OF FATTY ACIDS IN RABBIT-HEART SARCOSOMES. By Dr. W. C. Hülsmann	1153
REVERSAL OF TOXICITY OF 5-FLUOROURACIL AND 5-FLUORODEOXYURIDINE FOR *Candida albicans* BY PYRIDOXINE AND PYRIDOXAMINE. By Dr. M. L. Littman and Dr. T. Miwatani	1155
'GLYCEROL EFFECT' AND THE MECHANISM LINKING EXCITATION OF THE PLASMA MEMBRANE WITH CONTRACTION. By Prof. Masahiro Fujino, Toshio Yamaguchi and Keishi Suzuki	1159
MIXED SALIVA FROM SHEEP. By W. R. McManus	1161
DISSOCIATION OF FOOT-AND-MOUTH DISEASE VIRUS INTO ITS NUCLEIC ACID AND PROTEIN COMPONENTS. By F. Brown and B. Cartwright	1163
ANTITHROMBIN ACTIVITY OF NORMAL AND PATHOLOGICAL CEREBROSPINAL FLUID. By Dr. H. A. Gaertner, J. Lisiewicz and Dr. J. Caen	1164
EFFECTS OF ELECTRICAL STIMULATION OF RETICULAR FORMATION ON THE LACTIC ACID CONTENT OF THE RAT BRAIN. By Lj. Mihailović, Lj. Kržalić and D. Cvetković	1166
BIOSYNTHESIS OF CAROTENOIDS IN PURPLE BACTERIA: A RE-EVALUATION BASED ON CONSIDERATIONS OF CHEMICAL STRUCTURE. By Synnøve Liaaen Jensen, Dr. Germaine Cohen-Bazire and Prof. R. Y. Stanier	1168

(Continued overleaf)

SIMA

a unique source of INFORMATION on scientific instruments

There are two kinds of knowledge; knowing and 'knowing where to find out'. With science and technology developing so rapidly no one can hope to be fully informed on every worthwhile new technique or new equipment.

Here are three ways in which 'knowing where to find out' can help you:

(1) If you already know the answer to your problem but don't know who makes the most suitable equipment SIMA can tell you.

(2) If the answer to your problem depends on whether someone has already developed suitable equipment, SIMA will know if it exists and can tell you where to get it.

(3) If you just have a problem, SIMA can often tell you whether someone has already found the answer to it and has developed equipment to meet it.

Where do SIMA get their information?

From two sources. One is The British Scientific Instrument Research Association (BSIRA) which maintains a comprehensive library of catalogues. The other is the membership, consisting of over 180 companies engaged in all these fields of instrumentation:

OPTICAL & OPHTHALMIC	LABORATORY & ANALYTICAL
ELECTRONIC & NUCLEONIC	MEDICAL & X-RAY
ENGINEERING & INDUSTRIAL	KINEMATOGRAPHIC
NAVIGATIONAL & SURVEY	PROCESS CONTROL & AUTOMATION

The Scientific Instrument Manufacturers' Association could be your most valuable source of such information and you will find them very helpful on *any* question about scientific instruments.

the SIMA enquiry service

Over 180 manufacturers with one address

SCIENTIFIC INSTRUMENT MANUFACTURERS' ASSOCIATION OF GREAT BRITAIN

20, QUEEN ANNE STREET, LONDON, W.1.

Telephones—Secretariat Langham 4251 *Enquiry Service Imperial* 6000

This page has been donated by
UNICAM INSTRUMENTS LIMITED,
York Street, Cambridge.

LETTERS TO THE EDITORS

Page

GEOPHYSICS

Round-the-World Sound Waves produced by the Nuclear Explosion on October 30, 1961, and their Effect on the Ionosphere at Sodankylä.—G. Rose, J. Oksman and E. Kataja 1173

Potassium–Argon Age Determinations on Granite from the Island of Mahé in the Seychelles Archipelago.—J. A. Miller and J. D. Mudie 1174

Age Determinations of Rocks in the Lizard (Cornwall) Area.—J. A. Miller and D. H. Green . . . 1175

PHYSICS

Method of Measuring the Mössbauer Effect.—Dr. J. E. S. Bradley and J. Marks 1176

Investigation with the Electron Microscope of the Sublimation of Ice Crystals at Low Temperatures.—Prof. Tokio Nei 1177

RADIATION CHEMISTRY

Production of High Molecular Weight Products from the Irradiation of Methane with 4-MeV. Electrons.—R. W. Hummel 1178

CHEMISTRY

Valence State of Platinum–Alumina Catalysts.—R. J. Bertolacini 1179

Puzzolanic Activity and Semiconductivity of Fired Kaolin and Kaolinitic Clay.—A. K. Chatterji . . 1180

Sedimentation of β-Lactoglobulin (*A*,*B*) under Dissociating Conditions in Acid Solution.—Mrs. Lilo M. Gilbert and Dr. G. A. Gilbert 1181

Rotational Analysis of the $A^2\Pi$–$X^2\Sigma^+$ System of MgCl.—E. Morgan and Dr. R. F. Barrow . . 1182

BIOCHEMISTRY

Application of Atomic Absorption Spectroscopy to the Determination of Calcium in Saliva.—Ernest Newbrun 1182

New Penicillins Stable towards Both Acid and Penicillinase.—F. P. Doyle, A. A. W. Long, J. H. C. Nayler and E. R. Stove 1183

Cobalt Complexes of Proteins and Polypeptides.—Dr. Jake Bello and Helene R. Bello 1184

Formation of 2-Heptanone from Caprylic Acid by Spores of Various Filamentous Fungi.—R. F. Gehrig and Prof. S. G. Knight 1185

Isolation of a Cystine Derivative from Silk.—Dr. C. Earland and Dr. D. J. Raven 1185

Hydrolysis of α-Sulphoquinovosides by β-Glactosidase.—Isao Shibuya and Prof. A. A. Benson 1186

Interaction between Lysozyme and a Phospholipid of Neoplastic Tissue (Oncolipin).—R. Ferrari and S. Matracia 1187

Lysozyme from Human Milk.—Dr. Pierre Jollès and Jacqueline Jollès 1187

Purification and Concentration of Dye-Protein Conjugates by Gel Filtration.—Dr. W. George and Dr. K. W. Walton 1188

PHYSIOLOGY

Sodium Influx and Excitation in Uterine Smooth Muscle.—Prof. C. Y. Kao, Dr. D. Zakim and Dr. F. Bronner 1189

Effect of Some Smooth Muscle Stimulants on the Motor Response of the Isolated Guinea Pig Vas Deferens to Hypogastric Nerve Stimulation.—Dr. Nils O. Sjöstrand 1190

Non-steroid Inhibitors of Pituitary Gonadotrophic Function.—G. E. Paget, A. L. Walpole and D. N. Richardson 1191

Permeability of the Synovial Membrane to Glycoproteins.—Lars Sundblad, Dr. Eric Jonsson and Erik Nettelbladt 1192

Dietary Citrate and Hypomagnesæmia in the Ruminant.—A. W. A. Burt and D. C. Thomas 1193

Difference in Metabolism of Labelled Thyroxine between Thyrotropic and Adrenotropic Mouse Pituitary Tumours.—Dr. Sidney C. Werner, Eugene M. Volpert and Raul Grinberg 1193

PHARMACOLOGY

A Micromethod for the Determination of Bradykininogen in Blood Plasma.—Dr. Carlos R. Diniz, Ivan F. Carvalho, J. Ryan and M. Rocha e Silva . . 1194

Page

Biological Spectrum of Some New Tuberculostatic 1,3,4-Oxadiazolones with Special Reference to Cross-Resistance and Rates of Emergence of Resistance.—Prof. A. E. Wilder Smith and Hans Brodhage . . 1195

HÆMATOLOGY

Isolation of an α_1-Globulin from Human Plasma.—Dr. M. Steinbuch and Dr. J. Loeb 1196

Quantitative Sub-Groups of the *B* Antigen in Man and their Occurrence in Three Racial Groups.—Mary B. Gibbs, Col. Joseph H. Akeroyd and Julia J. Zapf . 1196

A Correlation between Surface Charge and Coagulant Action of Phospholipids.—Dr. A. D. Bangham . 1197

HISTOCHEMISTRY

Histochemical Examinations of Chymotrypsin-like Esterases.—Dr. David Lagunoff and Earl P. Benditt 1198

Histochemical Localization of Uridine Diphosphoglucose Dehydrogenase in Cartilage.—Dr. Károly Balogh, jun., and Richard B. Cohen 1199

IMMUNOLOGY

Mast Cell Lytic Antibodies.—Dr. I. Mota . . . 1201

PATHOLOGY

Apparent Induction of Malignant Tumours by Organ-specific Antiserum.—Prof. H. N. Green 1201

Susceptibility of Hamsters to the Oral Administration of *Mycobacterium johnei*.—N. J. L. Gilmour and J. G. Brotherston 1202

Behaviour of Protozoa under the Influence of the Synergetic Action of Polyethylene Glycol and Dyes.—Leopold Rejniak and Prof. Ludvik Komczynski . 1203

BIOLOGY

Molybdenum and Symbiotic Nitrogen Fixation by Alder (*Alnus glutinosa* Gaertn.).—Dr. J. H. Becking 1204

Œstrogen Content of Plants as a Function of Conditions of Culture.—Prof. R. Ferrando, Miss M. M. Guilleux and Mrs. A. Guerrillot-Vinet 1205

Incipient Dioœcy in Pimento.—G. P. Chapman and S. K. Glasgow 1205

Circular Streaming Patterns in a Slime-Mould Plasmodium.—Dr. Peter A. Stewart and Dr. Babette T. Stewart 1206

Production of Slime Substance in Mixed Cultures of *Rhodopseudomonas capsulatus* and *Azotobacter vinelandii*.—Prof. Azuma Okuda and Michiharu Kobayashi 1207

Seasonal Variation in Alginic Acid, Mannitol, Laminarin and Fucoidin in the Brown Alga, *Ecklonia radiata*.—C. M. Stewart, H. G. Higgins and Sue Austin . . 1208

Occurrence of *Phyllochaetopterus claparedii* McIntosh in the North-east Pacific.—R. Phillips Dales . . 1208

Oil Pollution of Marine Organisms.—Dr. M. George . 1209

MICROBIOLOGY

Solute Requirements for preventing Lysis of Some Marine Bacteria.—Prof. Robert A. MacLeod and Tibor Matula 1209

Dipicolinic Acid Content, Heat-Activation and the Concept of Dormancy in the Bacterial Endospore.—A. Keynan, W. G. Murrell and Prof. H. O. Halvorson 1211

Thermophilic Micro-organism of Uncertain Taxonomic Status from the Hot Springs of Tiberias (Israel).—David Kahan 1212

CYTOLOGY

Chromosome Preparation from Fresh and Cultured Tissues using a Modification of the Drying Technique.—M. Fox and Dr. I. M. Zeiss 1214

Culture of Human White Cells using Differential Leucocyte Separation.—James Hastings, Stanley Freedman, Orlando Rendon, Herbert L. Cooper and Kurt Hirschhorn 1214

Two Types of Ribonucleoprotein in the Nucleolus of Intestinal Carcinoma of the Newt following Injection of Herring-Sperm Deoxyribonucleic Acid.—A. Stolk 1215

PSYCHIATRY

Erythrocyte Cholinesterase-levels in Mental Patients.—Dr. George L. Ellman and Dr. Enoch Callaway . 1216

XUM

CHAPMAN & HALL

NUMERICAL ANALYSIS

By Z. KOPAL — SECOND EDITION

In this new edition of Professor Kopal's book, the main parts of the work are essentially unchanged, but developments such as the systematic applications of the operational calculus to numerical analysis—particularly those based on the use of rational (rather than polynomial) approximations—which were briefly dealt with in the first edition, now occupy a whole chapter. Other advances in the subject have also been taken into account in the revisions. 70*s. net.*

CHEMICAL KINETICS

By B. STEVENS

The first in a series of monographs on physico-chemical topics written for the university student reading chemistry as a secondary subject. Throughout the book an attempt is made to illustrate the various types of reaction by references to processes of natural and industrial significance. Wherever possible worked examples are based on published data of which much use has been made in selecting the problems collected together at the end of the book. *Illustrated.* 12*s.* 6*d. net.*

CRYSTALLIZATION: Theory and Practice

By A. VAN HOOK — A REINHOLD BOOK

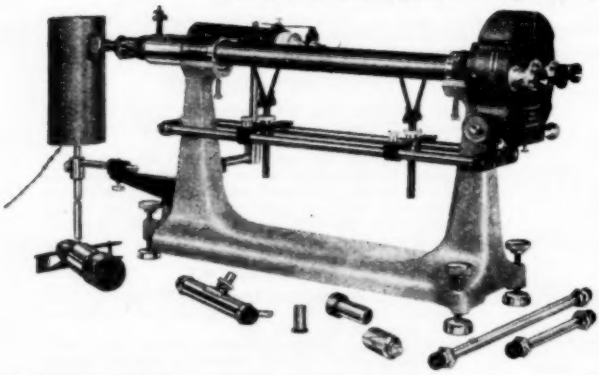
This excellent reference work presents recent theories of crystal growth. It discusses the principal processes, provides representative data on nucleation and growth, and describes the primary industries employing this unit operation. The author also gives the basic ideas important for an effective appreciation of the subject matter, and includes a historical sketch of crystallization. *Illustrated.* 100*s. net.*

37 ESSEX STREET, LONDON, W.C.2

PRECISION BS INSTRUMENTS

Polarimeter Model A

This instrument gives the highest degree of accuracy with divided circle on glass reading to 0·01 degree



Circle centre on ball bearings.
Easily removable trough.
Divided drum replacing verniers.
Stationary observation telescope.
Circle reader enlarging image of scale.
Slow motion adjustment always in action.
Suitable, with additions, for measurement of rotatory dispersion.

This polarimeter has been supplied in large numbers both in this country and abroad and has always been acclaimed as being of advanced design and workmanship.

Bellingham & Stanley Ltd.

DEPT. N, 71 HORNSEY RISE, LONDON, N.19
PHONE: ARCHWAY 2270

Sole distributors in U.K. for Schott Monochromatic Interference Filters

TECHNICAL AND SCIENTIFIC MAN-POWER IN BRITAIN

THE debate on technical and scientific man-power and research which was opened in the House of Lords on November 15 by Viscount Caldecote and Lord Shackleton drew a major speech from Lord Hailsham, the Minister for Science, in reply, but was also noteworthy for several constructive suggestions. Viscount Caldecote's purpose was to direct attention to the significance of making full use of the technical man-power of Britain and to the need to devote more resources in research and development in industry to enable exports from Britain to compete effectively in the changing conditions of world trade, and he was confident that if we made full use of the scientific factor we could lead in many more fields. He quoted with approval the view of Mr. S. P. Chambers that we could keep our balance of payments straight only by being among the world's leaders in developing new products and new processes. We had to export ideas and techniques as well as products.

Viscount Caldecote emphasized the importance of making full and proper use of trained man-power in order to maintain the quality of products, but he was highly critical of the magnitude of expenditure on home advertising compared with that on research and development. He was doubtful whether there is adequate co-operation between the universities and industry, and thought that there may be some duplication of research and development in the nationalized industries, particularly the service industries of transport, electricity and gas, where the emphasis should be on operational research. He suggested that further investigation is required into the possibilities of collaboration between industry and the universities and colleges of technology; but his suggestion for the appointment of a scientific committee akin to the Public Accounts Committee to investigate whether the nation's scientific resources are being adequately, properly and economically used seems to disregard the Advisory Council on Scientific Policy, in spite of a compliment he paid to the Council's work.

Viscount Caldecote also thought that the Research Associations should be expanded, though he agreed that some of the smaller associations might perhaps, with advantage, be amalgamated. He advocated larger grants, including capital grants, and an inquiry into the reasons why industrial support is so limited. He also pointed out the opportunities which the use of computers in management techniques may offer in gaining the full advantage of professionally trained staff, and he pressed the need to ensure that scientific and technically trained staff should have every opportunity to reach the highest positions.

By and large, Viscount Caldecote was concerned to avoid the waste of trained man-power. Lord Shackleton was concerned primarily to direct attention to the recent report of the Committee on Scientific Manpower on the long-term demand for man-power, of which he was both apprehensive and somewhat critical. While he recognized the reservations which the Committee expressed in the report, he seemed to think that the mere suggestion of a possible surplus of scientists and technologists was unfortunate and that the Committee had not pointed out in anything like sufficiently forthright terms the value of employing scientists and technologists outside positions that are strictly professional. Lord Marks referred particularly to the problem of the dissemination of information, which he regards as the greatest single difficulty, particularly among the small and medium-sized firms. Lord Fleck, however, thought that little is likely to be gained by further inquiry into the use of technical man-power, but that it is much more important to improve the training of technicians and apprentices, particularly to make better use of the higher grade of recruits who now enter on such training. He agreed, however, that we could make far better use also of the most highly trained technologists and scientists at the top; but for this he looked not to the new computer techniques and the like but to the use of the human sciences and especially psychology.

Lord Walston dealt more particularly with the character of the training which scientists and technologists should receive, and, while fully conscious that the Robbins Committee is examining the whole field, suggested that both the extent of university courses and the organization of the universities may require adjustment to meet the needs of to-day. Specifically, he suggested a rearrangement of academic terms which would in effect facilitate something like 'sandwich courses' (though he did not himself use the term) and in any event promote co-operation with industry for those studying social science as well as for students of engineering and science. He also advocated closer co-operation between the colleges of technology and the universities, and was anxious that the colleges should have the status that conferment of a degree rather than a diploma would give.

Lord Halsbury, who strongly supported the transfer of scientists and technologists across the economic structure, referred to their importance as administrators and on occasion as salesmen; he was highly

critical of the efficiency with which they are used, particularly in the Government service, and of the way in which administrative delays hinder technical progress. Lord Taylor was doubtful whether we are attracting sufficient of the ablest young minds into scientific and technical careers, attributing this largely to inadequate financial rewards as compared with those open to men who take up an administrative career. He stressed, however, the need for some degree of planning if the resources of Britain were not to be too diffused, but thought that free consultation and co-operation, particularly by using the statistics which planning produced, might be an effective answer.

Lord Hailsham, who replied, has within the past two months delivered three major addresses discussing the problems of science and Government in a free society, research in the universities, and again, in his Fawley lecture, to which Lord Taylor referred, science and Government. All these issues in some aspects or other were raised in the debate, and Lord Hailsham's reply naturally reflected a good deal of his conception of his office as indicated in those addresses. Nevertheless, his address in the House of Lords on November 15 was no mere repetition, and some of his comments were timely in a debate which once or twice seemed to reflect sensibility more than sense. Lord Hailsham commented that the demands on skill and knowledge for scientific and technical man-power in Britain are not exclusive demands, and are not substitutes for the knowledge of the arts and humanities or of the human sciences. He did not think that in any large respect an advance in science can any longer be obtained by getting out of the schools a much higher proportion of scientists as against humanists.

Lord Hailsham firmly defended the report of the Committee on Scientific Manpower, pointing out that the latest report was one of a series which went back as far as 1946 and that another survey would be made in January 1962. He maintained, moreover, that the prospects for any able young man or woman entering on a scientific or technological career are, and will remain, wider and better than ever before, and it would be a disaster if misinterpretation of the report made any such young people hesitate. He stated that the Government is seeking accurate information about the numbers of technicians in training, and agreed that it is essential that highly trained scientists and technologists should be adequately supported in this way. The University Grants Committee is also considering this aspect of the effectiveness of university research.

On the wider use of scientists and technologists outside strictly vocational fields, Lord Hailsham was in full agreement with other speakers. He referred to the critical shortage of teachers of mathematics, and in a passing reference to university research, in which he put the current research grants from the Department of Scientific and Industrial Research at a total of £2,970,000, expressed the view that the greatest need is to encourage research in industry. Industry, however, must in the end meet its own needs, and, turning to the Research Associations, although he agreed that the system could appropriately be re-examined and that there are several ways in which we could stimulate co-operative research, he was convinced that an approach which relied principally or primarily on State support is ineffective. In this connexion, supporting what Viscount Caldecote and Lord Marks had said about libraries and the dissemination of information, he suggested that even firms which are too small to conduct research themselves could well afford the modest subscription to their Research Associations which would make available to them so much information on other people's experience in research.

The difficulty here is, of course, that many such firms are not even aware of what they are missing, and that frequently they lack people capable of appreciating the significance for their own activities of current advances in science and technology. Here there might be a place for the scientific adviser, such as Lord Marks suggested; but though Lord Hailsham thought that the Department of Scientific and Industrial Research should encourage industrial research chiefly through the Research Associations and the work of its own research stations, he thought that there are two other promising possibilities. One is in the work of the Department's Economics Committee, established four years ago, in promoting investigations providing information essential in determining future policy. The Committee's preliminary work has led to more detailed and technical investigations of particular industries, two of which, on machine tools and shipbuilding, have been completed, while others are in progress.

Lord Hailsham believes that the machine tool project was outstandingly successful. It had effected a decisive change of atmosphere and stimulated a much greater interest by the industry in education. The design scholarship course sponsored by the industry at the Manchester College of Science and Technology is proving a great success, and schools of research are being developed in four institutions, to which the Department has already awarded research grants amounting to £170,000, and the industry has now established its own research association. In addition, the National Engineering Laboratory is making an impressive contribution in this field, and several projects for civil development contracts are under investigation.

The civil development contract is the other line of development which Lord Hailsham regards as particularly promising, and, quite independently of the work of the Economics Committee, the Department of Scientific and Industrial Research has been concerned with the general problem of large-scale development work and has been considering the use of civil development contracts to complement the work of the National Research Development Corporation. Lord Hailsham said that the Department has now been authorized to proceed with two major projects, and, if these projects succeed, we may have achieved a major break-through in the relations

between Government and industry in the encouragement of science. Early in his speech, Lord Hailsham had referred to the long period of time required to bring any plan to fruition—and especially in regard to education. The production of leaders in science may represent the better part of thirty years effort, and it may be for no more than a further ten that they are at the height of their powers, and perhaps fifty years before the application of their discoveries really begins. Nevertheless, he reiterated, in concluding, the importance of this connexion between education and man-power and productivity, and the ultimate dependence of progress on the devotion and creative inspiration of men of dedicated genius.

Although the main interest of this speech of Lord Hailsham lies in his reference to the work of the Department of Scientific and Industrial Research, and in the indication it gives of his attitude to the Research Associations, it reflects very clearly his consistent adherence to the principles which he laid down in his earlier address on science and Government. He adheres firmly to the idea of the maximum freedom for the scientist and technologist, whether in industry or in the university. The main task of the Government and its own institutions is to supplement and to stimulate, to correct the balance where necessary and to identify neglected areas in which some national effort is desirable. It was not appropriate on that occasion for Lord Hailsham to enlarge on the detailed implications of that policy or philosophy, though some of the questions asked might have tempted him to do so. He admitted, indeed, that the British system of training craftsmen and technicians needs a thorough overhaul, but otherwise made no reference to this aspect of the effective use of trained man-power. On the dissemination of information and the place of libraries and information services he made no comment; although it should be a prime concern of the Minister for Science, or any such philosophy as Lord Hailsham expounds, to see that both the National Lending Library for Science and Technology and the National Reference Library for Science and Invention are adequately conceived and planned as part of a national system, and that the resources placed at their disposal are adequate to the needs, not just of to-day, but of the decades immediately ahead. A firm adherence to the principles that fundamental research is best pursued at, or in close association with, the universities and that industrial research in the main is the business of industry, implies as a corollary that the Government has the responsibility to see that such ancillary services as library services, the supply and training of technicians and craftsmen, the provision of equipment, etc., for co-operative effort are generously adequate, and commensurate with national needs and technical potentialities rather than the limited horizons which may characterize a backward industry. It is in such respects that the Minister for Science has yet to convince some of those who are most ready to applaud his policy and his philosophy that his actions are worthy of his ideas and his vision.

CAPTAIN COOK'S VOYAGES

The Journals of Captain James Cook on his Voyages of Discovery

Edited by J. C. Beaglehole. Vol. 2: The Voyage of the *Resolution* and *Adventure*, 1772–1775. Pp. clxx + 1,021 (82 figures). (Cambridge: At the University Press, 1961. Published for the Hakluyt Society.) 120*s*. net.

ONE of the greatest explorers and discoverers of the eighteenth century, Captain James Cook, was responsible for important contributions to scientific knowledge. After Cook had completed his first voyage round the world in the *Endeavour* (1768–71), during which the transit of Venus was observed at Tahiti, with the support of the Admiralty he was soon able to pursue his further strategic plan of discovery in the southern hemisphere. By his own first voyage and by the work of others it had been proved that no southern continent of any magnitude could exist north of 40° south. The next step, therefore, was to see whether such a continent could lie south of the fortieth parallel. This Cook proposed to do by circumnavigation of the globe in high southern latitudes, taking advantage of the prevailing westerly winds. His aim was amply realized. Leaving London with two ships in July, 1772, he arrived back in July, 1775, after an absence of three years and eighteen days. Half-way through the voyage he had been parted from the *Adventure*, under Captain Furneaux, which returned to England alone. But he carried on with his plan in the *Resolution* and returned in good order, having lost but four of his crew, only one of these by sickness. As part of this remarkable feat of ingenuity and endurance, using New Zealand as a primary base with Tahiti as a secondary one, he made three major incursions into the antarctic and two into the tropical waters of the Pacific, each cruise yielding significant discoveries.

Crossing the antarctic circle for the first time in history, he succeeded on one occasion in getting farther to the south (71° 10′) than any other nautical explorer on record up to the present day has been able to achieve. He encountered a great deal of ice of various types which led him to many interesting observations. Though he did not establish the existence of the antarctic continent, and indeed considered it probable that no land would be found to the south of the ice, he did incline to the view that the ice extended in a solid body to the Pole.

Apart from the observations on ice and weather in these high southern latitudes right around Antarctica, Cook and his companions were responsible for various other important scientific achievements. The hypothesis of a great southern continent in the temperate zones, so persistently advocated by Alexander Dalrymple and others, was finally disposed of. In the tropical waters Cook, in pursuance of his plan, was able to confirm, systematize and extend in many particulars the discoveries of Pacific islands hitherto made. His observations on the Tonga group and on the New Hebrides were particularly full and significant, and his own geographical discoveries included Palmerston Island and Niue.

From the scientific point of view, one of the most important contributions of this voyage was undoubtedly the success of the chronometer for the establishment of longitude. Cook carried with him several of these instruments, not all of which were reliable, but the one which triumphantly proved

itself was that made by Larcum Kendall as a duplicate of the fourth instrument made by John Harrison. Known as "Mr. Kendall's Watch", this instrument received high praise from Cook and greatly facilitated the research of the astronomers aboard. The botanical and zoological records made by John Reinhold Forster and his son George, together with the anthropological and other observations of Cook and his companions, have now passed into the annals of the early scientific history of the Pacific.

This volume, like its predecessor (see *Nature*, 179, 390; 1957), is a model of editing. In the introduction to his first volume Dr. Beaglehole rather prematurely stated that the study of Cook's journals is more complicated for the first voyage than for the second. In the introduction to this volume he remarks rather ruefully that the more the textual problem for this volume is scrutinized, the more complicated it becomes, since there is no existing original version—properly speaking—of Cook's journal on the *Resolution*. The editor deserves, therefore, all the more congratulations for the meticulous way in which he has accomplished his task. The text primarily used for this volume is that of a holograph manuscript in the British Museum, written by Cook. This has been supplemented by material from another parallel manuscript, also by Cook, which he used as a draft for printing his own *Voyage towards the South Pole*, which appeared in 1777. Readers who have known the latter will recognize the great value of the present edition for the expansion of the text, and for the scholarly introduction and abundance of footnotes which assist a great deal in the interpretation. In addition, extracts from officers' records, including that of Furneaux; the journal of William Wales, the astronomer; papers from the Board of Longitude respecting instruments and their use; some letters by Cook; and an admirably annotated calendar of documents add much to the story of the voyage. Of particular human interest in the penumbra of scientific activity is the account of the abortive attempt by [Sir] Joseph Banks (afterwards president of the Royal Society) to assume a leading part in the voyage. In his introduction, Dr. Beaglehole seems to be rather hard on Banks. But his reproduction of Banks's own letter to the Earl of Sandwich, as First Lord of the Admiralty, makes it quite clear that Banks's concern for the size and accommodation of his own retinue weighed with him at that time more than a proper thought for the scientific objectives of the voyage. The volume is completed by an important series of maps and plans and many very effective illustrations, including a large number by Hodges, the official artist.

R. Firth

FLORA OF NEW ZEALAND

Flora of New Zealand

By H. H. Allan. Vol. 1: Indigenous Tracheophyta—Psilopsida, Lycopsida, Filicopsida, Gymnospermae, Dicotyledones. Pp. liv + 1085. (Wellington: Government Printer, 1961.) 105*s*.

THE publication of a new Flora of New Zealand is an event of supreme importance, and the work now available is a remarkable tribute to the industry and critical botanical sense of the author. Unfortunately, Dr. Allan did not live to complete his formidable task of compilation and elucidation, and this book, which includes all indigenous vascular plants except the monocotyledons, was most worthily finished and seen through the press by Miss Lucy B. Moore. It is thirty-six years since the last comprehensive descriptive account of New Zealand plants was published by F. T. Cheeseman, and in that period collectors and investigators have been most active in the study of the flora. Dr. Allan played a leading part in these researches and in field-work, and his wealth of knowledge of plants, botanical literature and the classic collections preserved in Britain equipped him admirably for the forbidding exercise of preparing a new Flora.

Students of the vegetation of New Zealand are well aware of the problems of unusual taxonomic complexity with which the flora of the country bristles. In many groups delimitation of acceptable taxa is notoriously difficult because of the high degree of polymorphy and the prevalence of natural hybridity. There is a wide range of habitats, and the comparatively small area of New Zealand has developed an extraordinary concentration of critical genera. Possibly, as has been suggested in the case of the north-temperate flora, some disturbance of the genetical balance during glacial periods has led to the intense and critical diversification which poses the multitude of taxonomic problems. Most informative, adequately referenced, supplementary notes throughout the work add greatly to its value and assist the reader to gain a better understanding of the many specific and generic complexities.

There is still ample scope for research, and Dr. Allan's work reveals most pertinently the many challenging features of the New Zealand flora. Within the compass of little more than a thousand pages he has produced a concise, convenient and eminently satisfactory Flora which will be of immense help in identifying New Zealand plants. Many beautiful New Zealand species have enriched gardens in other parts of the world, and horticulturists will certainly welcome this informative and valuable work.

There is an excellent chronological bibliography and, throughout, copious references are given to relevant literature. This is a work thoroughly to be recommended to all interested in the New Zealand flora—indeed, it is an indispensable tool.

George Taylor

SAMPLING AND ANALYSIS OF COMBUSTION PRODUCTS

Gas Sampling and Chemical Analysis in Combustion Processes

By Prof. G. Tiné. (AGARDograph No. 47.) Pp. xv + 94. (London and New York: Pergamon Press, 1961. Published for and on behalf of Advisory Group for Aeronautical Research and Development, North Atlantic Treaty Organization.) 42*s*. net.

THIS latest AGARDograph is devoted to a review of the experimental technique which forms its title, with particular reference to combustion phenomena of aeronautical interest. A proper appreciation of this subject involves a full understanding of many aspects of chemistry, aerodynamics and heat transfer, and it is rare to find an author with an equally detailed acquaintance of all three branches of science. It is clear from the introduction (Section *A*, 3 pages) that Prof. Tiné is well aware of the great importance of chemical phenomena in combustion processes, but

from the remainder of the book it is apparent that this is not where his own interests lie and that he is much more at home when discussing sampling (Section *B*, 53 pages) than he is when describing gas analysis (Section *C*, 31 pages). Indeed in the conclusion (Section *D*, 1 page), Prof. Tiné points out that "if sampling procedures were absolutely reliable, chemical analysis would raise no problem at all". This, in the reviewer's opinion, epitomizes one of the principal weaknesses in what is otherwise a useful and well-constructed book. The author gives the impression that chemical analysis is essentially straightforward and easy and thus—to quote but one example of this point of view—we read on p. 69 that "qualitative and quantitative analysis of alcohols and ethers is exhaustively described in most analytical textbooks", a statement that would surprise many expert analysts. The section on sampling, however, gives a masterly account of the various difficulties encountered in obtaining representative gas samples; it deals fully with such topics as the disturbance of gas flow by a probe and the problems involved in bringing about rapid quenching.

Prof. Tiné has made a noble effort to write a book which attempts to bridge the gaps which always tend to exist between different formal branches of learning. It contains a wealth of information, it is well produced and copiously illustrated with diagrams and tables and even if it is in some ways unbalanced in its treatment, it nevertheless provides the most useful and comprehensive treatment of the subject yet produced. The price is, however, even on present-day standards, high for a book of this length.

C. F. Cullis

THE ENZYMES

The Enzymes

Edited by Paul D. Boyer, Henry Lardy and Karl Myrbäck. Vol. 2: Purine and Pyrimidine Nucleotides and Phosphagens. Prosthetic Groups and Cofactors (Part A). Second edition, completely revised. Pp. xiii+479. 16 dollars

Vol. 3: Prosthetic Groups and Cofactors (Part B). Pp. xiv+497. 16 dollars.

Vol. 4: Hydrolytic Cleavage (Part A). Second edition, completely revised. Pp. xx+631. 18 dollars. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.)

THE second edition of *The Enzymes* is now expected to run to seven volumes, compared with the four volumes of the original edition prepared by Sumner and Myrbäck. Volume 1, on general principles, has already been reviewed in this journal (*Nature*, 184, 1759, 1959). Volumes 2 and 3 cover nucleotides, phosphagens, prosthetic groups and cofactors, and the chapters are numbered consecutively through these two volumes, since it was originally intended that this material should be covered in a single volume. Volume 4 is the first part of a survey of enzymes involved in hydrolytic cleavage; in this case also it has been necessary to extend to two volumes the treatment of a topic originally expected to occupy only one. These changes are sufficient evidence of the wealth of material available to the editors, and of the appropriateness of a new edition at this time.

At the end of Volume 3 is a chapter on "Enzymic Equilibria and Thermodynamics" which belongs to Volume 1, but was unfortunately delayed by Prof. Johnson's illness. A chapter of this kind, discussing basic ideas of equilibria, free-energy changes, *pK*'s, binding constants, and so on, is extremely valuable to the biochemist with little training in physical chemistry who wishes to understand the more quantitative work in the field of enzymology. On the whole this chapter succeeds in its purpose, although in places the treatment is perhaps too elementary, especially compared with the level of many of the chapters in Volume 1. For example, the importance of the *activity* of reactants in thermodynamic calculations might usefully have been stressed.

The subject-matter of Volumes 2 and 3 is primarily chemical rather than enzymological. There is a chapter each on nucleotides containing adenine, uridine, cytidine, and guanosine and inosine, and one on *N*-phosphorylated guanidines. The remaining 15 chapters come under the general heading "Prosthetic Groups and Cofactors", and include chapters on pyridoxal phosphate, folic acid, glutathione, flavins, coenzyme A, pyridine coenzymes, and lipoic acid, as well as a chapter on metal-enzyme interactions and four chapters on vitamins. The emphasis varies considerably from chapter to chapter, although it must be admitted that this may be due to the information available as well as to the idiosyncrasies of the authors. (It should be noted here that, as in the earlier volumes of *The Enzymes*, each chapter is written by a different expert.) The article on adenine nucleotides and pyrophosphate compounds by R. M. Bock is a useful general review of the main groups of enzymes acting on adenosine triphosphate, and a discussion of some of the mechanisms involved; those on uridine nucleotides by L. F. Leloir and C. E. Cardini, on cytidine nucleotides by E. P. Kennedy, and on guanosine and inosine nucleotides by M. F. Utter are largely metabolic, stressing the enzyme systems through which biosynthesis is brought about by these nucleotides. On the other hand, the articles on the pyridine coenzymes by N. O. Kaplan and on thiamine coenzymes by D. E. Metzler contain a much larger proportion of the pure chemistry of these compounds.

Two chapters seem to me to be of particular importance since they survey fields in which there is a good deal of recent work, and which are clearly ripe for an authoritative treatment. The first of these is on folic acid, which is excellently covered in 68 pages by J. C. Rabinowitz. The rather complicated chemistry is first set out, and then the main biochemical functions of folate derivatives are considered in turn: purine biosynthesis, serine biosynthesis, methyl group biosynthesis, and the metabolism of various amino-acids. Finally, the metabolism of folic acid itself is discussed. The other chapter referred to, by L. Jaenicke and F. Lynen, is on coenzyme *A*, and contains nearly 100 pages. This is particularly authoritative, since Lynen has himself been responsible for so much of the work which has made clear the metabolic importance of this compound. The chapter finishes with a useful table of 63 enzymes requiring coenzyme A or its derivatives. Another useful table is that given by A. E. Braunstein of enzymes requiring pyridoxal phosphate.

In view of the obvious pressure on space in this new edition, it seems doubtful whether it was necessary to include in Volume 3 separate chapters on vitamin K, vitamin E, ascorbic acid, and the visual

cycle. The role of vitamins K and E in oxidative phosphorylation is still a matter of some uncertainty and speculation, and cannot yet be discussed in terms of known enzymes. The chapter on ascorbic acid does in fact contain a good deal of enzymology, but treats the metabolism of the compound rather than its coenzyme function, which is not known.

Volume 4 of the present series is the first of three in which the main groups of enzymes and a number of individual enzymes will be surveyed. It deals with enzymes hydrolysing peptide, glycosidic and ester bonds. The chapters are arranged in groups, in each of which the first chapter is a survey of the field covered by those following. In the volume under discussion the 'survey' chapters are: "Peptide Bond Cleavage", by Emil Smith; "Other C–N Bond Hydrolyses", by J. E. Varner; "Cleavage of *O*- and *S*-glycosidic Bonds", by E. H. Fischer and E. A. Stein, and "Carboxyl Ester Cleavage", by D. K. Myers. This arrangement helps greatly to unify what might otherwise be merely a series of disconnected monographs. There is some evidence, too, of editorial efforts to avoid overlap between the chapters.

The general standard of this volume is very high, although inevitably the time taken to assemble manuscripts and produce such a volume has meant that in some cases recent developments are not included and the articles are somewhat out of date. Most of them, however, will probably remain authoritative sources for some time to come. It is not possible here to mention many of the 33 chapters; however, some stand out as giving a stimulating account of an enzyme, in which a large body of factual information is critically assembled, and used as the basis for a discussion of the mechanism of the enzymatic catalysis or the metabolic importance of the enzyme. Such chapters are useful reading for all enzymologists, whether or not their own work is connected with the enzymes under discussion. They include those on chymotrypsin and trypsin by P. Desnuelle; papain by E. L. Smith and J. R. Kimmel; α-amylases by D. French; β-galactosidases by K. Wallenfals and O. P. Malhotra; and acetylcholinesterase by I. R. Wilson.

The Enzymes is well printed on pleasant paper, and the volumes are strongly bound and are not unpleasantly large to handle. The whole work is too large to be regarded as a text-book, but as a reference book it should be on the shelves of every biochemist whose activities demand a reasonable knowledge of enzymology.

EDWIN C. WEBB

IDENTIFICATION, PROPERTIES AND USES OF INDIAN TIMBERS

Indian Woods

Their Identification, Properties and Uses. By Dr. K. A. Chowdhury and S. S. Ghosh. With the assistance of K. Ramesh Rao and S. K. Purkayastha. Vol. 1: Dilleniaceae to Elaeocarpaceae. Pp. liii + 304 + 30 plates. (Delhi: Manager of Publications, 1958.) Rs. 25.50 n.p.; 39*s*.

THE publication of a new reference book on Indian timbers is a notable event, for Gamble's *Manual of Indian Timbers* (1922) is now out of date and the more recent *Commercial Timbers of India* (1932) by Pearson and Brown omits many of the timbers that are now being exploited commercially. The volume under review is the first of a series of six, each dealing with the wood of 250–300 tree species that occur in the Indian sub-continent and are represented in the wood collection at the Forest Research Institute at Dehra Dun. The chief purpose of the book is to enable the timbers to be identified by characters that are to be seen with the naked eye or hand lens, and to assess the working qualities of the woods from their structural and physical properties. Brief notes on the habit and distribution of the species are given, together with some particulars of their strength, seasoning properties, durability, working qualities, supplies and uses. The descriptions of the woods are illustrated by photographs (× 10) of transverse sections. These illustrations, which are of excellent quality and well reproduced, add considerably to the value of the book. The scientific names and the trade and common names are given in separate indexes and there are appendixes dealing in tabular form with the nomenclature of species, classification of the woods by uses and by anatomical structure respectively.

After a valuable introduction of 53 pages, the main part of the book consists of descriptions of the woods under the botanical families to which they belong. For the sequence of families the authors have used the modified form of the Bentham and Hooker system adopted by Metcalfe and Chalk in *Anatomy of the Dicotyledons*. The volume now under review covers 20 families ranging from the Dilleniaceae to the Elaeocarpaceae and includes the important timber-yielding Dipterocarpaceae, more than 70 pages being devoted to this one family. At the end of each family there is an extensive bibliography.

The authors are at pains to dispel the popular conception that India is the potential source of large supplies of many diverse timbers. They emphasize that the number of timbers available in large quantities is not more than sixty or so, the most important being teak (*Tectona*), sal (*Shorea robusta*) and deodar (*Cedrus deodara*). Before 1939 the number of timbers handled commercially was 300–350, but about 500 were in use during the Second World War. Now the local exploitation of relatively unknown woods is becoming more common owing to the increased demand for timber resulting from industrial expansion and to the difficulties of transport.

In discussing the general question of how far wood structure can serve as an aid in taxonomy, the authors, in my opinion, take a somewhat pessimistic view. It is quite true, as the authors point out, that in many families the individual species, and sometimes the genera, are not easy to separate on the basis of wood structure. This, however, does not exclude the possibility of using wood characters for diagnostic purposes, and they are often valuable in determining the correct family relationships of genera the taxonomic position of which is uncertain from exomorphic characters alone. The great danger in the study of systematic anatomy is to rely too exclusively on wood characters alone without taking the histology of the rest of the plant into consideration.

The authors are to be congratulated on having produced the first volume of a most valuable book which cannot fail to be welcomed by botanists, foresters and wood technologists, and the publication of further parts will be eagerly awaited. It is a matter for regret that the quality of the binding does not do justice to a reference book of such undoubted importance.

C. R. METCALFE

Introduction à l'Electrodynamique Quantique

Par Prof. Daniel Kastler. (Travaux et Recherches Mathematiques.) Pp. xxxiii + 333. (Paris: Dunod, 1961.) 68 N.F.

THIS remarkable monograph has been written with the highly commendable aim of providing a common understanding between physicists and mathematicians, so far as the elements of quantum field theory are concerned. Whether this purpose has been achieved will probably be answered on the basis of the individual taste of any prospective reader. The present reviewer feels that the book will prove stimulating for the highly trained theoretical physicist, provided he has already acquired a fairly comprehensive familiarity with the customary formulation of quantum field theory.

The first two chapters and Appendixes *A*, *B*, *C* provide the general mathematical tools and notions: vector-spaces, Hilbert space theory, the Lorentz group and its representations, tensor- and spinor-calculus. A short chapter on the method of second quantization precedes the classical and quantized theory of the scalar Klein–Gordon field, Maxwell field and Dirac field (Chapters 4–9). Chapter 10 is devoted to a general discussion of the coupled Maxwell and Dirac fields, and the next chapter surveys briefly the formalism of interacting fields in general, including the *S*-matrix theory. (The relationship between the *S*-matrix and scattering cross-sections is established in Appendix *D*.) Chapters 12–14 show, by examples of Compton scattering, Möller scattering and pair annihilation the applications of the theory. The Dyson–Feynman technique and diagrams are discussed only in the next chapter. The book concludes with a review of external field problems.

The mathematical exposition is 'high-brow' and follows the pattern of best French tradition; but most physicists will probably find it difficult to rediscover within this work the familiar physical concepts they are looking for. P. ROMAN

Moon Atlas

By V. A. Firsoff. Pp. 32 + 7 plates + 2 fold-out maps. (London: Hutchinson and Co. (Publishers), Ltd., 1961.) 63*s*. net.

THE descriptive classification of lunar surface features to be found at the beginning of this atlas is a commendably lucid account of the intricacies of lunar topography. Unfortunately, the illustrations appearing later in this atlas do little justice to the lunar photographs now available. The four quadrant maps of the lunar surface, although artistically drawn, have the annoying property of including very few names; identifications have to be made via co-ordinates of individual craters. Page 22 consists of a very handsome set of photographs of progressive lunar phases but of restricted value. The relief map of the Moon, extremely ingeniously drawn, is of value to young astronomers with small telescopes; it is doubtful, however, whether these persons will be able to afford the cost of the atlas. The spherical projection photographs add little value to the atlas: although there are few such photographs available elsewhere, Firsoff's efforts have not succeeded in producing pictures of sufficiently good quality to contribute much to selenological thought. There are good reproductions of the Full Moon, the Moon's averted hemisphere, and the well-known Palomar Observatory Clavius photograph. It is misleading that these latter should be included in the section on spherical projection photographs. The *Moon Atlas* closes with Firsoff's personal map of the lunar grid system. The presentation (in three colours) of this chart leaves something to be desired and I personally prefer to use the small-scale reproduction appearing on the back of the dust cover. BRIAN WARNER

The International Dictionary of Physics and Electronics

Second edition. Pp. iv + 1355. (Princeton, N.J.; D. Van Nostrand Company, Ltd., 1961.) 27.85 dollars; 205*s*.

THE standardization of terminology in physics and electronics is not an attractive task. Some national and international bodies have tackled parts of it with fair success, but because they seek agreement between organizations with widely differing experience and outlook, their progress is often slow. This comprehensive dictionary, the work of a handful of scientists with an overwhelmingly academic background, is therefore very welcome, even though one can find some unevenness and lack of balance—most likely attributable to the speed of compilation—and some failure to draw sufficiently directly on the works of the established standardization bodies.

Three features distinguish the second edition of the dictionary from the first, published in 1956. First, a new introduction shows how classical physics retreated before experimental findings of the early twentieth century and how new theories filled the gaps created; parts of the old introduction, on units and dimensions, are retained under appropriate headings in the body of the book. Secondly, the inclusion of many new items lengthens the book by about 200 pages and, thirdly, multilingual indexes (in French, German, Spanish and Russian) are added.

The title 'Dictionary' understates the value of the book, for some entries would fully satisfy, and many partially satisfy, the title 'Encyclopædia'; the book is therefore educational as well as one of reference. The next edition will have many new fields to cover, such as the properties of space, lasers and the applications of low-temperature physics; if it must accordingly increase in size, might not a shorter or concise edition, prepared by cutting the length rather than the number of entries, find a market as well? J. R. TILLMAN

The International Zoo Year Book

Vol. 1, 1959. Pp. vii + 160. 19 plates. Edited by Dr. Desmond Morris and Caroline Jarvis. (London: Zoological Society of London, 1960.) 40*s*.

THE International Zoo Year Book fulfils a very long-needed want, but I feel it would be a great advantage if some check or verification could be made on the information given with regard to zoos—particularly in the Reference Section. To my knowledge there are many inaccuracies, especially with regard to details concerning the smaller zoos. This is my only criticism—otherwise I think the book is a very worth-while effort which will prove of great benefit to all zoological gardens.

It is a great advantage to have a list of the senior staff of the zoos of the world, together with their addresses, and also the various departments of zoology. The London Zoological Society should be congratulated on the way this book has been produced. G. S. MOTTERSHEAD

XUM

THE BETTER USE OF THE WORLD'S FAUNA FOR FOOD

THAT a large proportion of the world's population, especially in the technologically under-developed countries, suffers from either chronic or seasonal under-nutrition is well known. In many of these countries there is a continuous shortage of dietary calories, the ultimate effect of which is to slow down most human activities, with the apparent exception of the rate of reproduction. Not less serious than calorie shortage in its effect on the younger groups of the population and on pregnant and lactating women is the acute deficiency of dietary protein, which may occur with or without a calorie shortage, and is most obvious in its effects on the post-weaning child up to the age of about five years. Such protein deficiency is particularly marked in animal protein, usually richer in the amino-acids essential for growth and usually accompanied by a more valuable assortment of mineral salts, trace elements and vitamins than is vegetable protein.

The Freedom from Hunger Campaign of the Food and Agriculture Organization has as its objectives the improvement in quality as well as in quantity of food consumed in the existing hungry countries. It was therefore a most timely effort of the Institute of Biology to arrange a symposium, held during October 27–28 in the Royal Geographical Society's rooms, on "The Better Use of the World's Fauna for Food".

In the opening session, under Sir Julian Huxley's chairmanship, Dr. Francis Aylward (Borough Polytechnic, London), who has fairly recently returned from an extensive nutritional mission for the Food and Agriculture Organization in one of the under-developed countries, discussed the methods of assessment: (1) of protein requirements of individuals and communities; (2) of protein supplies actually available in a country; (3) of protein deficits on a regional, national and world-wide basis. The available statistical evidence of protein deficits, though not always as accurate as one could wish, was sufficiently convincing, apart from the additional evidence afforded by direct observations, by morbidity records and by mortality statistics. Though it was highly desirable to remove some of the uncertainty regarding the quality of the statistics and for further research on physiological requirements to be carried out, the need was even more urgent to use the scientific and technical knowledge now available in an intensive effort to improve indigenous protein supplies by the adoption of a more vigorous agricultural policy (in the widest sense, to include animal husbandry, fisheries and controlled utilization of wild fauna) and also by a more thorough application of modern food science to conservation and distribution of food.

"The Relative Merits of Plant and Animal Proteins" was the title of the paper given by Dr. A. E. Bender, whose important work on the assessment of the nutritional value of proteins and mixtures of proteins is well known to British nutritionists. Dr. Bender's contribution provided a useful epitome of the methods by which an assessment may be made of the comparative dietary values of different individual proteins, or mixtures of proteins. He related the figures obtained to needs at different ages and in different physiological conditions. The proteins of cassava, lapntain, breadfruit and sweet potatoes are inadequate for health of child or adult; those of rice, yams, millet and wheat can maintain the adult, but not the adolescent or the child. Some seed proteins, especially those of legume seeds (and particularly if two or more 'oil-seed' proteins, freed from oil, are mixed), will provide an adequate protein supply for all ages, if they constitute a sufficient proportion of the diet. The advantages and disadvantages of plant and animal proteins were compared, plant proteins being usually the cheaper, with a bigger yield per acre than animal proteins; recent attempts have been made to produce a cheap high-protein food mainly or even exclusively from vegetable sources (for example, 'Incaparina' in Central America). A mixture of animal with a poorer vegetable protein often gave a protein utilization result for the mixture almost as high as if the whole had been animal protein.

Religious prejudices in certain countries—not always under-developed countries—and local taboos more frequently handicapped the consumption of animal than of vegetable protein-rich foods, but the former were, for most people, tastier and more acceptable.

Though the answer to the protein-shortage problem in some countries might be intelligent vegetarianism, yet the advantages for the agricultural productivity of land of mixed farming, and the importance especially to the vulnerable groups of the population of having in the diet a reasonable percentage of protein food from animal sources, could not be overlooked.

Mr. T. J. Lansbury, in discussing potentialities of animal and particularly ruminant husbandry in West Africa, described the existing handicaps of absence of enclosure and of any intelligible system of land tenure, of 'night kraaling' with its consequent decrease in live-weight gain, of disease and particularly of the present of tsetse fly in many of the wooded areas, of unfertilized, mineral-poor pasture and semi-starvation of stock during long periods of drought, of catastrophic overstocking where cattle and even sheep and goats were regarded more as status symbols than as sources of food, being associated with bride price, ceremonial gifts and ritual sacrifice at traditional ceremonies.

The local cattle were nevertheless capable of good growth and production if adequate and controlled water supplies and the provision of dry-season supplementary foods could be organized. Two indigenous breeds of cattle, the n'dama and the West African dwarf shorthorn had considerable resistance to trypanosomiasis and were being introduced with advantage into certain tsetse-infested areas where well-distributed rainfall gave good grass production. The substitution of nomadic husbandry by mixed farming, enabling pastures to be suitably fertilized and fodder to be conserved for the dry season, entailed formidable sociological problems as well as wide dissemination of improved techniques.

Mr. Lansbury considered that pig and poultry husbandry probably offered fewer difficulties than cattle husbandry and that it should be possible to use modern knowledge to expand the pig and poultry industry rapidly and efficiently in West Africa, with great benefit to human nutrition.

The text of a paper by Prof. A. G. Bannikov, of the Academy of Veterinary Sciences, Moscow, on the

exploitation of the saiga antelope in the U.S.S.R. had been circulated, but unfortunately had to be discussed in the author's unexpected absence.

This antelope, which now occurs in increasing numbers in both European and Asiatic U.S.S.R. territories, was in the 1920's, as the result of uncontrolled, destructive hunting, almost extinct. Protective laws, introduced in 1919, intended merely to preserve the remnants of the species as a rare curiosity, had little effect at first, but in the 1930's and 1940's a rapid increase in numbers became evident and to-day the total saiga population in Europe is about 500,000, and in Asiatic U.S.S.R. about 1,500,000, mainly in the steppe plains and semi-deserts. It is a fertile ruminant, congregates in large and mobile herds and is now being systematically and rationally hunted, usually at night by organized groups of marksmen equipped with motor-vehicles with powerful headlights which attract the antelopes. Prof. Bannikov stated that the recovery of the saiga from near extinction to profitability is a vindication of the principles of intelligent nature conservation.

The use of an entirely different genus of animal for food was recorded by Dr. R. Orraca-Tetteh, who described the habits, collection and utilization in the diet of the giant African snail, *Achatina*.

Achatina, the largest living land mollusc, with, it was stated, a foot which when fully expanded might reach 30 cm. in length, is found in different parts of Africa, south of the Sahara. During the rainy season the snail population in the forests is often large. The animals are particularly active at night, browsing on tender leaves and fallen fruit, and growing rapidly. They are collected usually before sunrise and sent quickly to the market. They are killed by dropping snail and shell into hot water; the molluscs are then removed by hand. They may be eaten at once in soup or stew, or preserved for three months or more by smoking. The fresh samples contain about 16 per cent of protein, with a small amount of fat, some thiamine, riboflavin and niacin, and more than 70 per cent of moisture; the protein has a high content of lysine.

In parts of West Africa the snail is a very popular article of diet, and can be purchased cheaply during the rainy season.

Dr. L. Harrison Matthews discussed the problems and potentialities of wild-game ranching, which appears to be increasing, in Africa. The existing species of wild vegetivorous fauna in large areas in Africa have been established there long enough to become adjusted to their environment, making use of all the food resources, some by grazing, others browsing, others by eating the upper leaves of trees. The bio-mass of this wild fauna which is commercially exploitable is large, especially if natural predators are kept in check.

Most cattle ranchers coming into these areas in the past have destroyed or driven away the wild ungulates, regarding them as pests, though the cattle brought in have made less-efficient use of the vegetable food available.

The deliberate ranching of mixed wild fauna if they can be profitably cropped for human food is a commercial possibility that is now being exploited with some success. In Southern Rhodesia it is said to be more profitable than cattle ranching, while in the Republic of South Africa between 2,000 and 3,000 farmers are said to be stocking game, including such animals as zebra, wildebeest, impala, eland, kudu. The resistance of these animals to disease is greater than that of imported cattle. Transport and marketing problems in the sale of meat and hides are being solved. It was thought that once game ranching becomes commercially established, the extinction of the wild fauna concerned will be unlikely.

Efficient food processing preserves the nutritive value of the raw materials and yields a product acceptable to the consumer. P. L. Pellet and D. S. Millar, of the London School of Hygiene and Tropical Medicine, considered the processing and preservation of meat in under-developed areas from both these points of view. In tropical environments the slaughter of stock and the production of dressed carcasses either for immediate consumption or for preservation are fraught with temperature, and other, hazzrds. Further, the particular, and to Western eyes rather peculiar, food preferences of the local consumers have to be taken into account; for example, "To the Ghanian, the eating of, or rather the chewing of, meat is a pleasure to be taken leisurely, and consequently he regards tender flesh as low in value". Methods of preservation can seriously lower the nutritive value of any kind of meat or fish, but traditional preserved meats such as biltong, charqui, pemmican and kavurma are highly acceptable to the local consumers, and often showed little protein damage. If traditional methods of preservation were studied scientifically, many could be extended with advantage to meet the immediate protein needs of populations of under-developed countries. More sophisticated, modern methods such as refrigeration, canning, dehydration, vacuum contact drying, vacuum oil dehydration, production of meat powders, with the modern equipment necessary could be brought in as capital resources increased and indigenous food preferences and habits permitted.

The waters of the Earth (seas, lakes and rivers) occupy 75 per cent of its surface, but account for only 10 per cent of the world's food supply. The second day of the symposium was devoted to the problem of increasing food supplies from these sources. Mr. E. B. Parrish (Department of Agriculture and Fisheries for Scotland, Aberdeen) introduced the complex question of how to obtain maximum yield from traditional methods of fishing. The increasing intensity of fishing in certain areas such as the North Sea and north-west Atlantic has led to a decrease in the size and age of the fish caught and to a diminution in profitability of fishing. To prevent 'overfishing' of these areas, international conventions have been arrived at "for the investigation, protection and conservation of the fisheries" in order to make possible the maintenance of a "maximum sustained catch". Regulations for the fixing of catch quotas, for closed seasons, for closed fishing areas, for controlling the size at which fish could be caught by the fishing gear, for fixing of minimum landing-size limits, have been tried with mixed success.

In the conservation and management of fisheries large demands are made on scientific methods of investigating the population dynamics of the exploited fish stocks; these demands continue after regulations have been put into practice, to assess the responses of the exploited stock to them and give more precise adjustments of present fishing intensity to the maintenance of adequate future fish supplies.

Mr. F. D. Ommanney gave a fascinating account of sea fisheries in tropical areas. The poor gear and low status of the average tropical fisherman are slowly improving and in British dependent territories the work of fishery officers has led to major advances,

XUM

largely a result of fitting fishing boats with engines—outboard for the smaller and inboard for the larger craft, and partly by improved nets. At the same time, attempts have been and are being made, as a result of increased landings of fish, to reduce the price to the consumer.

Some Governments in British dependent territories, or those formerly dependent, have provided special fishing vessels for exploratory and pioneering work. Stations for fisheries investigations have been established in a few tropical countries, but owing to the vagaries of Government support have not been entirely successful. For the progress of tropical fisheries and the optimal use, in protein-hungry, maritime countries, of sea fauna for food, active and adequately supported research stations are urgently needed.

Improvements and trends of development in marine fishing methods and gear were the subject of a technical, well-illustrated contribution from Dr. J. Schärfe, of the Food and Agriculture Organization (Rome). Fish location and detection have been aided by aerial scouting and still more by fish-finding echo sounders and rangers. These need much skill and practical experience to use to full advantage. This technique is now firmly established for all medium and large fishing vessels in the developed countries. Synthetic fibres stronger and more elastic than natural fibres are being used very widely for nets and lines, and have inspired new ideas in the design and operation of fishing gear and methods.

Larger stern-trawlers of up to 3,000 h.p. with freezers, cold storage and in some cases manufacturing plant for oil and fish meal can now go quickly to distant grounds and remain away from home ports for extended periods. Fishing fleets exist with boats specialized for searching, catching, factory work, transport, hospital and recreation facilities. Mid-water trawling with echo sounding is a modern line of development which is likely to have far-reaching results.

Electrical killing or stunning of fish in confined waters has been experimented with with some success and may have a future even for sea fish.

Mr. K. F. Vaas, of Yerseke (Netherlands), pointed out that fish culture in freshwater and brackish ponds was both an ancient and a very widespread craft. *Tilapia* was cultivated in ponds in Egypt before 2,000 B.C., and a text-book on fish culture was written in China in 475 B.C. Large yields of fish per acre of water surface are obtainable under favourable conditions—the Grand Lac in Cambodia with an area at high water of 10,000 km.^{2} is said to produce 100 kgm. fish per hectare.

Tropical ponds give better yields than those of colder climates, but much depends on 'management' in the shape of fertilizers or the introduction of fish foods of various kinds. Fish culture may be combined with animal husbandry, the dung fertilizing the growth in the ponds of fish food such as diatoms, plants and crustaceans. Production of fish from inland waters has been calculated to be some 18 per cent of the total world catch.

The culture of marine molluscs and crustaceans was reported on by P. R. Walne, of the Ministry of Agriculture, Food and Fisheries, Fisheries Experiment Station, Conway. In the United Kingdom, shellfish form only 2·5 per cent, in Japan 19 per cent, in the Netherlands 28 per cent, and, rather surprisingly, in the United States 30 per cent of the total fish landings. Oysters and mussels are slow growing, the mussel requiring four years, and the oyster five, to reach marketable size. The meat of the adult oyster or mussel contains some 80 per cent of water and 9–13 per cent of protein, that is, roughly 50 per cent or more of protein on the dry weight. Oysters are probably the most carefully cultivated crop obtainable from the sea. In recent years mechanical methods, including special dredges, are being used for removal of the bivalves to new grounds from time to time, and for final harvesting. Such dredges attached to power-driven boats can work economically on quite low population densities. By this means both the mussel and the oyster stock in Holland is moved several times to assist growth and reduce starfish depredation.

Mr. Walne considered that an important future step in oyster husbandry should be what has been done with practically all agricultural plants and animals, namely, to increase the rate of growth and quality of the stock by breeding and selection.

Some recent advances in the processing of fish were described by Dr. D. L. Nichol, of the Department of Scientific and Industrial Research, Humber Laboratory, Hull, in relation to the more efficient use of this source of food.

'White' fish gutted, washed and stored in ice at sea become stale after 12–15 days, and if distant water voyages are to last longer than the average of 18–20 days (4 days each way to the fishing grounds) apparatus is needed for deep freezing at sea at least part of the catch. In trials carried out in 1956, this method of preservation of whole fish has been proved successful and more trawlers are being fitted out with the necessary deep-freeze equipment.

Deep-frozen fish is now being distributed in various forms to consumers. It is necessary to keep the temperature down all along the distribution chain, and this is not always realized, as the difference between a block of frozen fish at − 20° F. (a temperature at which no deterioration occurs in eight months) and + 20° F. (a temperature at which deterioration occurs within a month) is not obvious to the operator.

Herring processing presents different problems from that of white fish, as herring fishing is seasonal and the disposal of gluts may arise. Gluts of herrings are usually transformed into oil and fish meal, the latter being utilized in the manufacture of animal feeding stuffs and increasingly in tinned food for domestic pets.

White fish meal prepared from surplus or condemned fish and from fish offal is manufactured in the United Kingdom and abroad in substantial quantities for use in animal feeding stuffs. Fish flour, a deodorized and almost flavourless powder usually made from whole fish, is proving acceptable as an addition to human diet in certain protein-deficient regions.

A paper on the possible farming of the sea from Sir Alister Hardy concluded the symposium. He discussed the possibility of improving the production of plankton and hence of the fish that ultimately depend on plankton for food either by fertilization of limited or confined areas of sea with phosphates and nitrogen or by causing up-welling of the deeper layers of sea-water which are richer in nutritive salts than the upper layers. He also mentioned methods for diminishing the enormous loss of fish that occurs in early life and the possible transporting of young fish from crowded to less-crowded areas.

Recent research has shown that invertebrate competitors of fish on the sea bed, such as starfish, are much more important in limiting the potential supply

of food available to fish than was formerly realized, and that at least in limited areas such pests might be greatly reduced in numbers by specialized trawling.

To summarize, the following are among the main considerations that emerged from the symposium:

(1) The rapid rise in world population makes it doubly important that there should be increased production and much better utilization for human food of land fauna, both domestic and wild, and of sea fauna.

(2) The potentialities of such increased production are large if present agricultural, animal husbandry and fisheries knowledge is effectively applied, and if the utilization of wild animals is conducted intelligently and under adequate control; these potentialities are immense if sufficient research effort is directed towards the further acquisition of knowledge in these fields, particularly in that of marine biology.

(3) The rate of progress in improving the protein status of malnourished populations will depend largely on indigenous decisions to correlate their farming and their fisheries policy with indigenous nutritional needs, and actively and continuously to stimulate the intelligent knowledgeable local production of fish, flesh and fowl.

The proceedings of the symposium will be published by the Institute of Biology (41 Queen's Gate, London, S.W.7).

H. D. KAY

FIFTH INTERNATIONAL CONGRESS OF BIOCHEMISTRY

THE fifth International Congress of Biochemistry, organized by the U.S.S.R. Biochemical Society and sponsored by the International Union of Biochemistry and the Academy of Science of the U.S.S.R., was held in Moscow during August 10–16, under the presidency of Academician A. I. Oparin. The scientific sessions were held in the imposing building of the University of Moscow which stands on the Lenin Hills and overlooks the historic city. The number of registered participants, about 4,500 together with an additional 1,000 or so guests of the Organizing Committee, was larger than any previous congress, and it seems that each congress, from the first, which was held in Cambridge in 1949, to the present one in Moscow, attracts more and more workers who are interested in one or many aspects of the broad subject of biochemistry.

The congress held its opening ceremony in the impressive Palace of Sports of the Central Lenin Stadium and the session closed with a masterly lecture on "The Structure and Function of Subcellular Particles", by Prof. D. E. Green (United States). Unfortunately, poor projection facilities reduced the enjoyment of many participants who were not fortunate enough to be seated near the front of the huge lecture hall. The plenary session was followed by a most enjoyable concert given by the Moscow Symphony Orchestra.

The scientific programme which followed included a series of symposia, and between two and three thousand original contributions given under the auspices of twenty-eight sections. The main congress symposia were held in the large assembly hall of the University or in auditoria of ample seating capacity and were well attended throughout. The symposia covered the following subjects: (1) Biological Structure and Function at the Molecular Level (president, M. F. Perutz, Great Britain); (2) Functional Biochemistry of Cell Structures (O. Lindberg, Sweden); (3) Evolutionary Biochemistry (S. Ochoa, United States); (4) Molecular Basis of Enzyme Action and Inhibition (P. Desnuelle, France); (5) Intracellular Respiration: Phosphorylating and Non-Phosphorylating Reactions (E. C. Slater, Holland); (6) Mechanism of Photosynthesis (H. Tamiya, Japan); (7) Biosynthesis of Lipids (G. Popják, Great Britain); (8) Biochemical Principles of Food Industry (E. Pyanowski, Poland). The main speakers in these sessions were allowed up to forty minutes to develop their subject and the subsequent speakers fifteen minutes, a half to three-quarters of an hour being set aside at the end of the session for discussion. The time proved to be adequate, and the discussions were usually lively. The arrangements for simultaneous translations in these meetings were excellent.

Among the many important communications given in the symposia Dr. Britton Chance's demonstration of a new effect in the field of intracellular respiration predicted on theoretical grounds some years ago by Sir Hans Krebs, namely, the reversal of the direction of electron transfer in isolated mitochondria occurring on the addition of adenosine triphosphate and leading to a reduction of pyridine nucleotide was particularly noteworthy; as were Prof. A. L. Lehninger's report on a preparation of hitherto unrecognized components of the chain of oxidative phosphorylation and their relation to the swelling and contraction of mitochondria, and Prof. Lynen's account of the advances he and his colleagues had recently made in the understanding of the role of (+)-biotin as a coenzyme of carbon dioxide fixation and transcarboxylation. Prof. E. P. Kennedy (United States) gave an outstanding lecture on lipid biosynthesis. There were, of course, many other new and interesting aspects of fundamental problems presented at the congress, but the author, in common with other participants, was able to hear only a small part of them. Pre-prints of the main congress lectures and of the contributions to the symposia were available to participants before the meeting.

Probably the largest part of the scientific programme was associated with the activities of the twenty-eight sections which held their meetings and discussions in nearly as many rooms scattered throughout the very large University building and in the Departments of Physics and of Chemistry situated a few minutes walk from the main building. Two of the Sections, those concerned with Immunochemistry and the Biochemistry of Malignant Tumours, held their meetings in the Sternberg Institute of Astronomy, which was unfortunately about a 15-min. walk away from the site of the main congress activities.

Each section had its own interpreter, and language difficulties were thereby reduced to a minimum. Abstracts of the short communications were available in the form of a separate book, and where the text

was submitted in Russian, an English translation was also provided.

A Russian version of the whole of the congress proceedings is to be published by the U.S.S.R. Academy of Science and a corresponding publication in English (10 vols.) will be made by the Pergamon Press, Ltd. These volumes are in course of preparation.

There was a useful display of scientific apparatus originating from fourteen countries, and an attractive exhibition of modern books dealing with all aspects of biochemistry and published in many different countries.

A significant achievement, admittedly not directly a part of the congress, was the appearance of the report of the Commission on Enzymes, of the International Union of Biochemistry. This document, which is the first official attempt to put enzyme nomenclature on an international basis, is the work of a group of acknowledged experts under the chairmanship of Dr. Malcolm Dixon of the University of Cambridge. It makes an outstanding and much-needed contribution to modern enzymology.

Most of those attending the congress were accommodated in the student quarters of the University or in two of the largest hotels in Moscow, Hotel Ukraine and Hotel Mockba. The problems which arose through accommodating so many visitors in so short a time were finally sorted out, although only after some participants had suffered considerable inconvenience. It was generally agreed, however, that once one had found the accommodation for which one had originally asked, the standard of comfort was all that could be desired. Transport between the University and hotels was well maintained throughout the congress by an excellent bus service. The hosts were most generous with their hospitality and Academician Oparin gave a series of most delightful receptions to the participants in the symposia. There was a series of interesting tours arranged by Intourist.

The participants from the Commonwealth much appreciated the kindness of Sir Frank Roberts, the British Ambassador in the U.S.S.R., who held a reception so that British participants might entertain their Russian colleagues who had given so much time and made so much effort to make the congress the scientific success it undoubtedly was.

Plans for the next international congress are by now under serious consideration, and it must be in the minds of the organizers that it will be attended by an even greater number of scientists than were present in Moscow this year. It is clearly impossible for any one person to attend more than a few of the sessions, and it is also evident that ten minutes for the presentation and discussion of each ordinary communication is inadequate. It might, therefore, be worth considering whether a more satisfactory procedure for use at future congresses would be to restrict the number of speakers, without limiting the number of papers communicated by title and circulated in the form of abstracts. A limitation of this kind would also reduce the number of simultaneous sessions, which, it is generally agreed, are far too many at large international congresses in which there are only four or five days to present and discuss all the papers submitted by the participants.

Perhaps to-day the long notice required to publish and pre-circulate abstracts of the scientific contributions makes it unlikely that anything really new will be announced at an international congress. Probably the most valuable function of such a meeting is to bring workers together to take part in symposia and to engage in informal discussion, where they can estimate their progress or thrash out their differences or misunderstandings in small rooms or quiet corners which are set apart for such gatherings, but which are within easy reach of the main meeting rooms and where light refreshments would be available.

The fifth International Congress in Biochemistry was the largest congress yet held in the U.S.S.R. and it was obviously well supported by the Russian Government. It is generally recognized that the more biological aspects of science in the U.S.S.R. have up to the present received far less attention than have the physical and engineering sciences; but the impression gained was that there is a growing awareness of this relative neglect, and that there was an awakening to the fact that biochemistry and other biological subjects must in future receive the support they so justly deserve and need.

The closing plenary session was especially noteworthy for the outstanding and informative lecture on "Proteins, their Function and Structure" given by Prof. F. Sorm (Czechoslovakia). This was followed by a number of short addresses of appreciation by delegates from several countries, after which the president closed the proceedings and formally announced that the next congress would be held in New York in July 1964. W. T. J. Morgan

ATTENTION AND IN-ATTENTION IN NEUROPHYSIOLOGY*

By L. GOLDIE† and Mrs. J. M. GREEN
Institute of Psychiatry, Maudsley Hospital, London, S.E.5

RECENT advances in neurophysiological techniques have enabled objective evidence to be readily and reliably obtained during long recordings, and cortical and subcortical electrical events to be examined.

Despite this there has been little increase in the understanding of the working of the human brain as it relates to mental experience. Much experimental work in the past has centred on the animal brain. Transposition of the theories derived from such studies to the special problem of the human brain has resulted in the formulation of certain philosophical devices to overcome complexities by narrowing the observations within certain controllable limits.

But philosophical issues must inevitably be subjected to the test of practical experiments. The Dualists, in supporting a metaphysical theory of reality, regarded the mind as having an independent

* Substance of a paper read at the Ninth Latin American Congress of Neurosurgery during a symposium on the "Physiological Basis of Mental Activity", October 1961.

† Present address: The Fountain and Carshalton Group, Queen Mary's Hospital for Children, Carshalton, Surrey.

existence, and since this was considered to be beyond the reach of natural science discounted it in formal experiments. The psycho-physicists, exemplified by Fechner, did much the same thing when they narrowed the limits of their experiments by attempting to exclude the effects of subjective factors, and then concentrated on the properties of the stimulus which they expressed in terms of mathematical formulæ. Pavlov went even further and chose to disregard the subjective factor altogether, assuming physiology to be a sufficient explanation of the mind. Psychophysical parallelism, on the other hand, while acknowledging essentially human manifestations to be accompaniments of physical activity in the brain, considered them to vary concomitantly without one affecting the other and therefore to be outside the scope of experimental method.

These manœuvres have resulted in any effect of mental life being disregarded, and have led to the emphasis being placed on the physical attributes of stimuli. But the problems imposed are the very essence of conscious human being and to simplify them is to destroy the very quality that it is hoped to study. Such writers as Helmholtz[1], Wundt[2] and James[3] were not restricted by metaphysical considerations and evinced an open-minded acceptance of the available facts in the context of the fundamental issues raised, discussing them comprehensively and elegantly. By the same token Sherrington[4] and Adrian[5] reiterated the unanswered problems impressed on them by their experiments and observations on human subjects. Thus Adrian comments that "though one brain may look like another, what it does in response to a stimulus may be quite different because it depends on all the brain has done since it was first in action". This central problem remains and has become of paramount importance in neurophysiology, where inconsistent results of experiments and some routine findings on human subjects have not been fully explained by a physiological stimulus-response association[6–11].

In this article the attempt has been made to examine aspects of the fundamental psychological processes of attention and apperception, considering also the terminology that may usefully be applied and then to relate these to physiological changes. Some common-place phenomena such as may be seen in routine and experimental neurophysiological recordings are considered and used for illustrative purpose to support the suggestion that results, which on superficial examination appear to be random, may be better understood if, when causal analysis is attempted, the mental no less than the physical characteristics of the human subject are accepted and used as data.

An elementary instance of this approach is provided by the psychogalvanic skin response, the variation of which in occurrence was apparent during interviews recorded on this unit. (Electrophysiological experiments performed by us utilized two small rooms, one for the patient and one for the apparatus, linked by a one-way screen. The observer in the patient's room was kept informed of events in the instrument room via an intercommunicating system linked to a deaf-aid earpiece. The electroencephalogram and appropriate peripheral ancillary recordings were made during interviews. The sessions were recorded on magnetic tape and these were correlated with the electroencephalogram, which could also be marked by the observer using a marker-button.) It was observed that incidental door bangs from the corridor usually elicited a response in subjects and patients when they were waiting for the observer to enter the room. During the interviews, however, though psychogalvanic skin responses were occurring in conversation, they were rarely seen in relation to door bangs (the latency of the response to a known stimulus having been established). When a response did occasionally occur to a door noise, it was found on cursory examination that the interview material usually indicated that at such times the entry of another person (such as an unauthorized person or a nurse with an injection tray) would, for example, have been a frightening or embarrassing possibility. Here it appears that the assessment of the physical properties of a stimulus is alone insufficient to account for the behaviour of these responses.

The question of adaptation was considered and in the experimental situation when sound and light flash stimuli were repeated for some time without instructions, responses were produced that tended to 'habituate', that is, to decrease and disappear. However, when subjects then performed a series of tasks each one commencing when the stimulus sounded, the response was shown to behave differently and to alter with each repetition of the stimulus. These tasks involved mental imagery and concentration, and there were experiments with simple hand or finger movement and variations with touch. From such experiments it appeared that when the physical conditions of an experiment, including the stimulus, remained the same, variations in the response could be causally related to mental activity. We are reminded by Adrian[12] that "the method of experiment is to relate the stimulus to the resulting response but this ignores the fact of attention to the stimulus"; the crucial consideration in this sense and in our experiments hinges on the meaning of the word 'attention'.

When recognizing attention or mental activity one should perhaps first consider the distinction between the external world of objective reality and the reflexion of this in the internal world of psychical reality. The description of a stimulus by an observer must necessarily have a limited connexion with the response, for the stimulus occurs in the external world of the subject while the response is related to his internal world. The response to the stimulus will therefore be governed by the 'significance' that the stimulus has for the individual subject at any particular moment.

In another context this is very obvious when one considers a child reacting with a temper tantrum to something we may think is innocuous. In terms of the external world the child's reaction is inappropriate, but the situation as depicted in the inner world of the child may explain the behaviour: "In a tantrum the violence of the physical struggle being due to internal changes such that [he] becomes blind and deaf to external reality[13]". This is further illustrated by an experiment of Sharpey-Schafer *et al.*[14], who investigated the mechanism of acute hypotension from fear or nausea in a series of patients submitted to anæsthesia for dental operations. He demonstrated that in four out of five subjects in whom acute hypotension occurred during the administration of gas, arterial pressure fell before the mask was placed on the face. The dental surgeon and anæsthetist did not recognize that severe hypotension was present and were apt to call this state 'a nice quiet gas'. The result was therefore attributed to the wrong 'cause' and had tradition not ordained that the

XUM

patients be lain flat the outcome would have been irreversible. The author comments: "It is suggested that emotional stimulation of the heart beat and a fall in cardiac filling pressure caused virtual emptying of a ventricular chamber during systole which fires the afferent mechanism of the faint reflex".

In electrophysiological experiments Hernández Peón[15] recorded the occipital responses to single light flashes using subcortical electrodes and found that the response amplitude became larger with the increased intensity of the light. Keeping the brightness constant he then suggested alterations in intensity and demonstrated that the occipital responses to light flashes showed a greater change in line with suggestions than with objective variations in light intensity. It may be said that subjective factors influenced some facet of 'attention' paid by the patient to the light flashes and this determined the variations in the occipital responses. Hence these reactions can be better comprehended when they are thought of as the result of object relationships in the internal world. This reasoning must also apply in the tantrum and dental situations.

A comparable instance was observed by us[16] during an intensive study of a case of sensory reflex epilepsy. A clear-cut electrical wave-form occurred in the left parietal cortex when the patient rubbed the right side of his face, which was also associated with the production of a fit, and the experiments showed that this could also be effectively produced by psychological means alone. During experimental sessions on this patient it was demonstrated that the electrical response, besides occurring when he rubbed his face, appeared when he made the rubbing movements without touching his face (the patient was hypnotized). It was confirmed statistically that this psychological stimulus was just as effective for the evocation of the wave-form as actual rubbing. The null hypothesis was tested that this response could have happened by chance alone and was shown to be highly improbable. The electrical response also appeared when he anticipated rubbing his face, and it occurred when he was asked to imagine rubbing his face, or when he imagined having a fit: this was demonstrated in the laboratory and while recording directly from the brain exposed at operation. At other times the occurrence of the electrical activity during conversation appeared to be related to certain topics.

Consequent to these findings another sample of recordings was examined to determine the frequency of similar episodes occurring during the resting state when stimuli were not deliberately being given. It was established that during a period of 15 hr. 35 min. recording time, the electrical response appeared only on ten occasions unrelated to any known stimulus, but on forty occasions was temporally related to happenings that had been noted on the record (for example, intermittent noises, including the telephone and people entering the room, etc.). It was then shown that during a much shorter period of 3 hr. 28 min. recording time, under experimental conditions, the response appeared on thirty-five occasions in relation to known psychological stimuli, statistically exceeding anything that could occur by chance alone. These instances show that a causal sequence may be established using data that at first sight appear to be random or spontaneous.

In another experiment performed on the same patient in the hypnotic state an attempt was made to produce an anæsthesia of the right cheek. Loss of sensation was suggested, and when the right face was touched by the observer there was no change in the electroencephalographic record. On another occasion, however, the suggestion was worded differently and the patient was told that the right side of his face was "frozen and insensitive". Contrary to what was expected the electrical response was immediately evident at high amplitude when this area was touched, but did not similarly occur when he was touched elsewhere on the face (Fig. 1). After this session the patient volunteered the information that he had been surprised and impressed to find that his right face had felt intensely cold. It was thus evident that his interpretation of the suggestion had differed from that which had been intended and that he had in effect focused 'attention' on this area of the face instead of away from it. Here the only clue to the unexpected results lies in the information the patient provides regarding his interpretation of the suggestion. It appeared that the factors governing the result included the direction of attention, the exclusion of other matters, and possibly the amount of effort involved in the task.

The evidence presented establishes that a relationship can be demonstrated between mental activity and physiological changes. But to understand something of this problem it is first necessary to examine aspects of the process of attention and then to use descriptive terms that have practical application. William James[3] and Adrian[17] describe attention as consciousness being occupied by one thing at a time; to Sherrington[4] attention was but a further application of the rule that "unlike reflexes have successive but not simultaneous use of the common path. Expressed teleologically, the common path, although economically subservient for many and various purposes, is adapted to serve but one purpose at a time. Hence it is a co-ordinating mechanism and prevents confusion by restricting the use of the organ, its 'minister', to but one action at a time. . . . The resultant singleness of action from moment to moment is a keystone in the construction of the individual whose unity it is the specific office of the nervous system to perfect. The interference of unlike reflexes and the alliance of like reflexes in their action on their common paths seem to lie at the very root of the great psychical process of 'attention'."

The application of attention to one thing at a time necessitates the simultaneous withdrawal of attention from everything else that is not attended to. This is described as 'in-attention', and produces a descriptive hiatus. In an attempt to resolve this difficulty Freud's concept of mental energy[18] is put forward for consideration. "The Pre-conscious has at its disposal for distribution a mobile cathectic energy, a part of which is familiar to us in the form of 'attention'." This idea of a constant amount of mental energy was first formally proposed by Cabanis[19]: "Sensibility seems to behave like a fluid whose total quantity is fixed and which whenever it pours into one of its channels in greater abundance becomes less in others" . . . and it was referred to again by Janet. Using this concept some resolution of the semantic problem is possible. An object engaging our attention could be said to be endowed with a special significance and meaning, and result in a redistribution of cathectic energy. While this energy is focused on one object, there is simultaneously a reduction of energy available for other objects. When an object is weakly cathected, another object may readily sup-

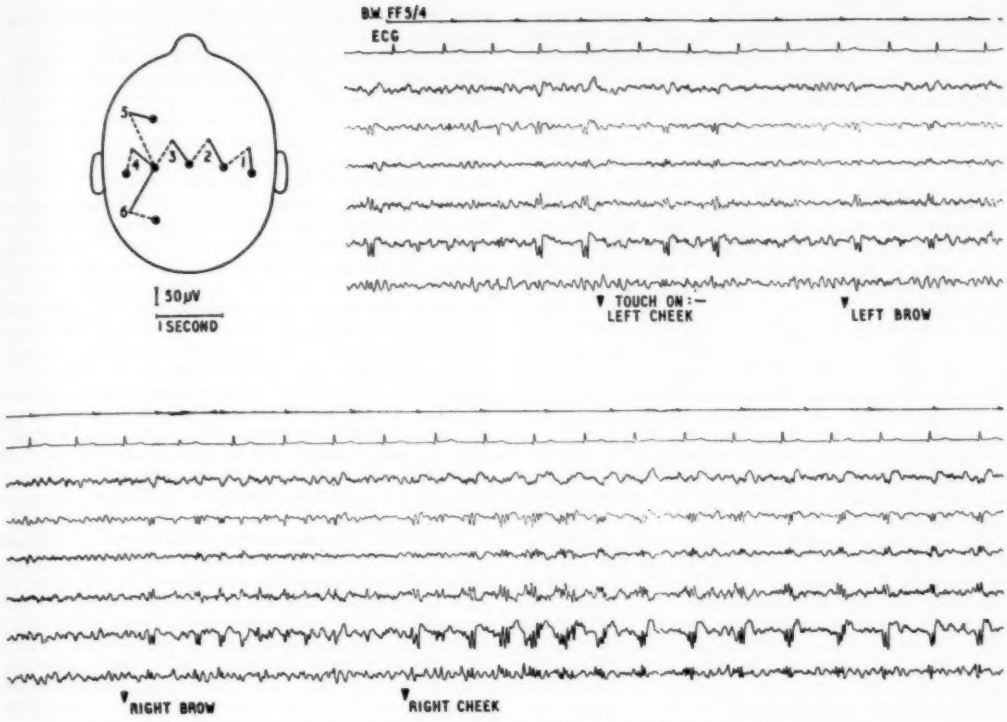


Fig. 1. Time constant, 0·1 sec.; high frequency, 25 per cent ; amplifier sensitivity, 7 mm. for 50 μV. Input

plant it: in common parlance it may then be said that we are 'easily distracted'.

This formulation may be applied to lambda-wave investigations[20], where it was an unequivocal finding that in the majority of control subjects tested, light, eye movement and some facet of psychological 'set' were all necessary for the evocation of lambda-waves in the occipital cortex when looking at a picture. It appeared that interest, effort or the focusing of attention in a particular direction were necessary, that is, the picture had to be 'cathected'. The problem, however, remains as to whether recorded changes may be due to 'attention' or 'in-attention' or due to the process of redistribution of cathectic energy. Adrian[17] in 1934 and afterwards, by his exhaustive examination of the alpha rhythm on himself and his co-workers, makes the consideration of such mental processes inevitable. He speaks of closing the eyes with the automatic withdrawal of attention from visual phenomena . . . "we try to see in the dark: when the subject does not expect to see anything the rhythm persists". "A problem that contains the whole attention even with the eyes shut will block the rhythm, it will return as soon as the 'attention' wanders". "The rhythm is stopped if the whole attention is involved. Perception of light does not affect it: perception of pattern or the *attempt* to perceive it does".

Characteristically the alpha rhythm of the human, while varying enormously from subject to subject, when fully developed, remains of constant form for the individual. The phenomenon has much in common with that seen in the water beetle[17], and is presumably connected with basic biological activities necessary for survival, though in human beings the added cerebral organization obscures and complicates the connexion. In man, vision is the dominant sense and the only one that can be voluntarily controlled, and it is perhaps not without reason that the alpha rhythm is most easily influenced by the act of opening and closing the eyes. It may also be modified by psychological factors, the examination of which inevitably concerns us with the central process of attention. Opening the eyes must involve at a primitive level the 'taking in' of visual information from the external world which confirms or denies the possibilities arising as a result of sensory input from other modalities. Similarly, closing the eyes may shut out visual stimuli arriving from the external world, but also has the effect of increasing dependence on other sensory information which is interpreted in the light of past experience. With such an act these interpretations become prepotent because they now lack the possibility of visual checking with reality. These considerations therefore make it necessary to try to examine the physiological changes in terms of a relationship with the mental counterparts of physical acts. This possible relationship was found to occur in the case of sensory reflex epilepsy already described where the mental counterpart of the physical act was shown to be as effective in evoking the electrical response as actual rubbing of the face.

That psychological factors can influence the alpha rhythm behaviour may be well illustrated by the occasions of its appearance and non-appearance and its fluctuations, during recorded interviews or silences, when the eyes were kept open or closed. The significance or meaning that the situation had for

the subject, and also the amount of continued 'force', 'effort', or 'cathectic energy' that was invoked appeared to be but two of the factors that could inhibit the alpha rhythm. This was particularly prominent with visualization.

It was, however, notable that the rhythm could be both evoked or inhibited when speech started or stopped. This was surprising, since one might expect the rhythm to be inhibited on the commencement of conversation and to reappear on its cessation. Furthermore, when it was shown that one subject, who had a sensitive high-amplitude alpha rhythm which was classic in behaviour, was attending closely to a discussion which at one stage involved the description of a biological film, his alpha rhythm continued unabated. Not only was the subject kept deeply involved but when electronic sound stimuli were introduced during the discussion they failed to effect any alteration in the alpha rhythm or to elicit a psychogalvanic skin response within 3 sec. of the stimulus. From these observations and some experiments involving mental tasks which produced inconsistent results it appears then that attention cannot be considered or discussed in psychologically unsophisticated terms.

That mental processes could be related to physiological functions was recognized by Darwin[21] when, for example, he wrote . . . "Blushing is not only voluntary, but the wish to restrain it by leading to self-attention actually increases the tendency" . . . and . . . "the reddening of the face from a blush is due to the relaxation of the muscular coats of the small arteries, by which the capillaries become filled with blood, and this depends on the proper vaso-motor centre being affected". Reflecting on the mental state that produces blushing he wrote . . . "It is not the simple act of reflecting on your own appearance, but the thinking what others think of us which excites the blush". A brief consideration of deceptively simple phenomena therefore yields complexities: paying attention to a person will not of itself produce a blush without the intervention of affective factors. In Darwin's example it may be said that an affective response has been induced by a mental image which is endowed with certain attributes: here the evocative stimulus is not the object in the environment but the feelings we have about it or its supposed intent.

With the above presumptions we may with profit re-examine other situations which remain causally enigmatic. Of the epilepsies, the 'reflex' type is perhaps most pertinent since there is an apparently specific stimulus response association. Apart from the case of sensory reflex epilepsy already quoted it was found in other cases that the causal connexion was not a simple one and that the emotional substrate had to be considered. The clearest demonstration of this was provided by three patients suffering from musicogenic epilepsy where the music alone was shown not to be directly and consistently responsible for the fits. In our experiments the metronome sounds and gramophone-record music alleged by the patients to precipitate fits were presented. Apart from rare occasions when fits occurred, the patients reported that the stimulus had not produced the feeling that occurred when a fit was imminent. Conversely, fits occurred when there was a marked emotional response to the music, and this was often accompanied by an electroencephalographic change. This observation has already been noted by Critchley[22], Shaw and Hill[23] and others. Therefore it can only be the psychological import of the stimulus that produces the evocative effect.

The function of the affective labelling of experience governing behaviour and the direction of attention may be illustrated by a patient with an obsessional neurosis which in this connexion was a case of singular interest (details of which are to be published elsewhere). In response to a specific word or allusions to it, the patient's blood pressure rose from 185/124 to 230/155. He became extremely distressed, restless and anxious, and tried by every means to avoid the conversation which was for him disturbing. This patient was seen again after he had been subjected to a leucotomy and the rise in diastolic and systolic pressure was then even more rapid and was still sufficiently alarming to prevent mention of the word. By contrast there was, however, a striking clinical change, for there was a noted absence of observed or reported experience of anxiety, though the pressor response was even more marked. There were no attempts by the patient to remove himself from the stimulus as he had done before leucotomy because his protective mechanism utilizing anxiety against a dangerous rise in blood pressure had been interfered with. Focusing of attention cannot simply be governed by the conscious discernment of certain properties of the environment, and the word presumably had a particular unconscious meaning for the patient and was attended to in a particular way because of the subjective experience of anxiety. This is but an exaggeration of the normal process whereby objects are attended to and distinguished one from another by their affective value, differentiation being ultimately impelled by pain/pleasure consideration. This complex and indirect connexion with an objective stimulus could also be said to apply to the cases of reflex epilepsy.

Our discussion has raised certain theoretical questions inherent in any work on conscious human beings.

Starting with some considerations that arise out of limited neurophysiological studies it has been necessary to re-appraise the fundamental question of attention and the approach to this problem. The waking state, and therefore consciousness, is conceived of as a theoretic state which is inferred when discrimination is exercised in attending to internal as well as external stimuli; the switching of attention from one thing to another further implies an executive function performed by the ego which organizes information that is not in consciousness (pre-conscious) into a probability hierarchy awaiting entry into consciousness. Einstein[24] concerned himself with the stimulus response problem when he asked, "What, precisely, is 'thinking'? When, at the reception of sense-impressions, memory pictures emerge . . . (and) It is by no means necessary that 'concept' must be connected with sensorily cognizable or reproducible sign (word); but when this is the case thinking becomes by means of that fact communicable. . . . For me it is not dubious that our thinking goes on for the most part without use of signs (words) and beyond that to a considerable degree unconsciously" (note, his brackets).

Adrian[25] said that "research has shown how information gets into the brain but not how the brain reacts to it". It is apparent from physiological studies that sensory input is always the same, from whatever organ, so that it is the arrival point that determines whether we see or hear, and contact with reality is necessarily retrospective. But to determine what in consciousness we see or hear,

we have to be concerned with the psychological aspects of attention or inattention based on representations that are an amalgam of sensory input, past experience and symbolization. Psychical reality consists therefore of very individual representations. The focus of attention is the product of these factors and in the process the peripheral receptors may be affected[26]. The relating of psychical to physiological events is therefore necessary in neurophysiological experiments. The usual causal equation of objective stimulus and objective response lacks the interposition of the psychical stimulus with the mental and emotional reactions. This leads us to consider the terms that have to be used to describe such observations. Generalizations based on these accounts require the use of psychoanalytic conceptions, to avoid the too-ready assumption that there is a simple psychological event directly accounting for a physiological change.

Thus we are led to suggest that there are complementary points of view that are both necessary for the understanding of perceptual processes. In such experiments we have two observers: the experimenter with his objective recordings, and the subject who is an introspective observer who attempts to report on the content of consciousness, that is, that on which attention is focused. Since the latter's account is retrospective, he necessarily uses complementary terms to describe a state of mind, at the same time being subject to a continuous experience. Philosophical issues concern us less than the epistemological difficulties of dealing with two such streams of information and the attendant problems of communication.

This work was supported by a grant from the Ford Foundation, at the Institute of Psychiatry, The Maudsley Hospital, London.

Thanks are due to Prof. Denis Hill, Mr. Murray A. Falconer and Dr. J. P. Dewsbery, who made available patients used in this work.

[1] Helmholtz, H. V., *Handbuch der physiologischen Optik*, third ed. (Voss, Hamburg and Leipzig), trans. edit. by Southall, P. C. (Optical Society of America, 1924).
[2] Wundt, W., *Grundzuge der physiologischen Psychologie*, second ed. (Wilhelm Engelmann, Leipzig, 1880).
[3] James, W., *The Principles of Psychology*, **1** (Macmillan, London, 1891).
[4] Sherrington, C., *Integrative Action of the Nervous System*, 234 (Cambridge Univ. Press, 1947).
[5] Adrian, E. D., *The Analysis of the Nervous System*, Sherrington Memorial Lecture. *Proc. Roy. Soc. Med.*, **50**, 991 (1957).
[6] Goldie, L., *Brit. J. Med. Psychol.*, **32**, 124 (1959).
[7] Goldie, L., and Green, J. M., *Nature*, **187**, 952 (1960).
[8] Goldie, L., and Green, J. M., *Nature*, **189**, 581 (1961).
[9] Goldie, L., and Green, J. M., *Nature*, **191**, 200 (1961).
[10] Goldie, L., and Green, J. M., *J. Psychosomat. Res.* (in the press).
[11] Goldie, L., and Green, J. M., *Epilepsia* (in the press).
[12] Adrian, E. D., *Archiv psychiatric und Zeitschrift ges. Neurologie*, **196**, 482 (1958).
[13] Isaacs, Susan, *Intern. J. Psychoanalysis*, **21**, 280 (1940).
[14] Sharpey-Schafer, E. P., Haytor, C. J., and Barlow, E. D., *Brit. Med. J.*, 878 (Oct. 1958).
[15] Hernández-Peón, R., and Donoso, M. (personal communication). *Abst. Rep. Proc. First Intern. Cong. Neurol. Sci.* (Brussels), **3** (Pergamon Press, London, 1959).
[16] Goldie, L., and Green, J. M., *Brain*, **82**, Pt. 4, 505 (1959).
[17] Adrian, E. D., and Matthews, B. H. C., *Brain*, **57**, Pt. 4, 385 (1934).
[18] Freud, S., "Interpretation of Dreams", in the Standard Edition of the complete *Psychological Works of Sigmund Freud*, **5**, 593 (Hogarth Press, London, 1953).
[19] Cabanis, P. J. G., *Oeuvres completes de Cabanis*, **3**, 153 (Bossange, Paris, 1824).
[20] Green, J. M., *EEG. Clin. Neurophysiol.*, **9**, 691 (1957).
[21] Darwin, C., *Expression of the Emotions in Man and Animals* (John Murray, London, 1872).
[22] Critchley, M., *Brain*, **60**, 13 (1937).
[23] Shaw, D., and Hill, D., *J. Neurol. Neurosurg. Psychiat.*, **10**, 107 (1947).
[24] Einstein, A., *Albert Einstein—Philosopher Scientist. Library of Living Philosophers*, edit. by Schilpp, Paul Arthur (Tudor Publishing Co., New York, 1951).
[25] Adrian, E. D., *Brit. Med. Bull.*, **3**, 1 (1945).
[26] Hernández-Peón, R., Guzman-Flores, C., Alcarez, M., and Fernandez-Guardiola, A., *Acta Neurol. Latino-America*, **3**, 1 (1957).

AMINO-ACID SEQUENCE OF HORSE HEART CYTOCHROME *C*

Peptides released by Digestion with Chymotrypsin

THE only part of the amino-acid sequence of cytochrome *c* which has been established so far is the region to which the hæm is attached[1] through thioether bonds between the two cysteine residues in the peptide chain and the side-chains of the protoporphyrin[2]. Since the conformation of the entire protein has a profound influence on the enzymatic and physico-chemical properties of the prosthetic group[3], an understanding of the interplay of structure and function in cytochrome *c*, as well as of its reactions with other components of the terminal oxidation chain, must be based on an accurate knowledge of the entire protein. As a first step towards the elucidation of the structure of cytochrome *c*, a study of its complete amino-acid sequence was undertaken. The present communication discusses the peptides recovered from chymotryptic digests of horse heart cytochrome *c*.

Methods and Amino-acid Sequences of Peptides

Horse heart cytochrome *c* (ref. 4) was purified by twice chromatographing on 'Amberlite *IRC*-50' (ref. 5). Chromatographic fraction I, the enzymatically fully active protein, containing 0·45 per cent iron, was denatured in 60 per cent ethanol. The lyophilized denatured protein (100 μmoles) was digested at room temperature for 29 hr. with a twice-recrystallized chymotrypsin preparation which exhibited no detectable tryptic activity. The mixture was maintained at *p*H 7·8 with an automatic titration apparatus, and 2 per cent by weight of chymotrypsin was added three times during the course of the digestion. The complete digest was chromatographed at 40° on a column of 'Dowex 50 × 2' 3·7 cm. × 150 cm. with volatile pyridine–acetic acid buffers by applying a linear gradient of *p*H and ionic strength from 0·2 *M* at *p*H 3·1 to 2·0 *M* at *p*H 5·0. The peptides in each fraction were purified further by various combinations of electrophoresis and chromatography on paper. The amino-acid composition and the purity of each peptide isolated were determined by analysing suitable aliquots after acidic or enzymatic[6] hydrolysis on the automatic amino-acid analyser of Spackman, Stein and Moore[7]. All purification steps were guided, and their efficacy monitored, by use of the two-dimensional electrophoresis-chromatography technique of Ingram[8]. The yields of peptides were calculated from the analytical data on the final

Table 1. Peptides obtained from Horse Heart Cytochrome *c* by Digestion with Chymotrypsin

No. – *C**	Sequence	Yield (per cent)
XIX*a*	Acetyl (Asp,Gly) Val.Glu.Lys.Gly.Lys.Lys.Ileu.Phe (*N*-terminal peptide)	94
XXXIII*a*	Val.Glu-NH_2.Lys.Cys.Ala.Glu-NH_2.Cys.His.Thr.Val.Glu.Lys.Gly.Gly.Lys.His (hæm)	60–70
XXV*a*	Lys.Thr.Gly.Pro.Asp-NH_2 (Leu,His) Gly.Leu.Phe	63
XXVI*a*	Gly.Arg.Lys.Thr.Gly.Glu-NH_2.Ala.Pro.Gly.Phe	62
XIII*a*	Thr.Tyr	38
XX*a*	Thr.Asp.Ala.Asp-NH_2.Lys.Asp-NH_2.Lys.Gly.Ileu.Thr.Try	44
XVIII*b*	Lys.Glu.Glu.Thr.Leu.Met.Glu.Tyr	47
XXIV*a*	Leu.Glu.Asp-NH_2.Pro.Lys.Lys.Tyr	49
XVIII*a*	Ileu.Pro.Gly.Thr.Lys.Met	47
XIV*a*	Ileu.Phe	53
XXVIII*a*	Ala.Gly.Ileu.Lys.Lys.Lys.Thr.Glu.Arg.Glu.Asp.Leu (Ileu,Ala) Tyr	55
IV*a*	Leu	19
XXIII*a*	Lys.Lys.Ala.Thr.Asp-NH_2.Glu (*C*-terminal peptide)	44

* Peptides are designated by the prefix *C* to indicate their origin from a chymotryptic digest. The fraction number indicates the sequence of elution from the ion exchange resin.

products and thus represent only minimal values, in some cases probably as much as 25–30 per cent less than the actual yields, because of variable losses during purification and because the original column chromatographic fractions were pooled to obtain as pure fractions as possible.

The amino-acid sequence of each peptide was established by combinations of chemical and enzymatic methods appropriate to each particular case. The chemical procedures used were the dinitrophenylation technique of Sanger[9] for the identification of amino-terminal residues and the Edman method[10] for the sequential identification of amino-acids in peptides. At each step of the Edman procedure, in addition to the chromatographic identification of the phenylthiohydantoin of the *N*-terminal amino-acid, an aliquot of the residual peptide was hydrolysed and its amino-acid composition quantitatively established. Digestion with leucine aminopeptidase[11] or with carboxypeptidase *A* often permitted identification of *N*-terminal and *C*-terminal residues or short sequences, respectively. When digestion of peptides with endopeptidases, such as trypsin and papain, was performed, the resulting derived peptides were purified and the composition and sequence of each one were established by the methods already cited.

The sequences of the chymotryptic peptides isolated from horse heart cytochrome *c* are listed in Table 1. Except for the hæm peptide, the peptides given in Table 1 are only the longest peptides isolated; in most cases shorter peptides were obtained in much higher yields than the 'overlapping' long peptides. The yields cited represent the sum of the yields of the shorter and longer peptides included in a given sequence.

In the column chromatography of the original chymotryptic digest, the main hæm-containing peptide could not be eluted from the 'Dowex-50' resin. It was prepared from another digest by column chromatography on 'Amberlite *IRC*-50'. Since the enzymatic hydrolysis in this experiment was not carried to completion, the hæm peptide obtained was larger than the hæm peptide of which the amino-acid sequence is given in Table 1 and which represents the minimal chymotryptic 'core' of cytochrome *c*.

The peptides recovered from the chymotryptic digest represent the entire amino-acid complement of horse heart cytochrome *c*, the composition of which was determined by analysis of acid hydrolysates (Margoliash, E., Hill, R. L., Kimmel, J. R., and Schmidt, W. R., paper in preparation): Try, 19 Lys, 3 His, 2 Arg, 3 Asp, 5 Asp-NH_2, 10 Thr, 9 Glu, 3 Glu-NH_2, 4 Pro, 12 Gly, 6 Ala, 2 CySH, 3 Val, 2 Met, 6 Ileu, 6 Leu, 4 Phe, 4 Tyr. The amide distribution was determined on enzymatic hydrolysates.

N-terminal and *C*-terminal Sequences

The first peptide listed in Table 1 behaved abnormally. It contained three lysyl residues and one each of glutamic acid and aspartic acid and would, therefore, be expected to have an overall positive charge at near neutral *p*H values; however, on electrophoresis at *p*H 6·5, it behaved as a neutral compound, indicating that either one positive charge had been eliminated or an extra negative charge added. The lysyl residues all had normal unsubstituted ε-amino groups, since digestion with trypsin resulted in practically quantitative yields of all four peptides expected. Moreover, the lysine residues were recovered as ε-dinitrophenyllysine after treatment of the peptide with fluorodinitrobenzene. On the other hand, all attempts to identify an *N*-terminal residue failed, indicating that there was no free α-amino group. Thus, the peptide could not be digested with leucine aminopeptidase at ratios of enzyme to substrate fully effective for all other peptides; no *N*-terminal residue could be recovered after prolonged dinitrophenylation; two successive steps of the Edman procedure did not release any phenylthiohydantoin derivative and did not result in any change in composition of the residual peptide. Gas chromatography of a suitable acid hydrolysate established the presence of acetic acid, and it was concluded, therefore, that the amino-terminal α-amino group was acetylated. This finding explains the abnormal charge of the peptide. Incubation of the peptide in methanolic 1 *N* hydrochloric acid gave a mixture in which only aspartic acid and glycine could be identified as amino-terminal residues by the Edman procedure, thereby placing these residues at the amino-terminal end.

Since this peptide had an acetylated α-amino group, it must represent the amino-terminal sequence of cytochrome *c*. This assignment of position was fully substantiated by the work of Kreil and Tuppy and when the entire amino-acid sequence of the protein was determined (see following communications). Similarly, acetylated amino-terminal α-amino groups have been shown to occur in other proteins, for example, melanophore-stimulating hormone[12], in tobacco mosaic virus protein[13], in egg albumin[14], and in turnip yellow virus protein[15].

The amino-terminal residue of horse heart cytochrome *c* has been reported by one of us (E. M.) to be histidine[16] and by another group of investigators to be arginine[17]. Both these claims are erroneous, since the three histidyl residues and the two arginyl residues in the molecule have now been located in the interior of distinct chymotryptic peptides that must

be located in the portion of the protein carboxyl-terminal of the hæm.

The last peptide listed in Table 1 has a carboxyl-terminal glutamic acid. Clearly this residue could not represent a possible point of attack for either chymotrypsin or trypsin. Moreover, no longer sequence of which this peptide was a part could be found. The last peptide listed in Table 1 was assigned, therefore, to the carboxyl-terminal position in the protein. This conclusion is in accord with the results of Titani, Ishikura and Minakami[16] who identified glutamic acid as the carboxyl-terminal residue of horse heart cytochrome *c* by the hydrazinolysis method. Similar findings have also been obtained by Kreil and Tuppy (see following communication).

In summary, the amino-acid sequences of the peptides obtained by digestion of horse heart cytochrome *c* with chymotrypsin have been determined and these peptides account for the amino-acid composition of the protein. There is no free α-amino group in the protein. The amino-terminal residue occurs as the acetyl derivative. The amino-terminal chymotryptic peptide has the structure: Acetyl (Gly, Asp) Val.Glu.Lys.Gly.Lys.Lys.Ileu.Phe. The carboxyl-terminal residue is glutamic acid with the terminal chymotryptic peptide having the sequence: Lys.Lys.Ala.Thr.Asp-NH_2.Glu.

While this work was in progress, we learned from Prof. H. Tuppy that an investigation of the tryptic peptides of cytochrome *c* was under investigation in his laboratory; it is a pleasure to acknowledge the co-operative exchange of information which has led to simultaneous and joint publication of results. We are indebted to our associates, Dr. A. Light and Dr. J. R. Kimmel, for their help and advice. This investigation has been aided by research grants from the National Institutes of Health, U.S. Public Health Service.

A complete description of our work will be submitted for publication in the *Journal of Biological Chemistry*.

E. Margoliash*
Emil L. Smith
Laboratory for the Study of Hereditary and Metabolic Disorders
and the
Departments of Biological Chemistry and Medicine,
University of Utah College of Medicine,
Salt Lake City, Utah.

* Present address: McGill-Montreal General Hospital Research Institute, Montreal.

[1] Tuppy, H., and Paléus, S., *Acta Chem. Scand.*, **9**, 353 (1955).
[2] Theorell, H., *Biochem. Z.*, **298**, 242 (1938); *Enzymologia*, **6**, 88 (1939). Paul, K.-G., *Acta Chem. Scand.*, **5**, 389 (1951).
[3] Margoliash, E., Frohwirt, N., and Weiner, E., *Biochem. J.*, **71**, 559 (1959).
[4] Kellin, D., and Hartree, E. F., *Biochem. J.*, **39**, 289 (1945).
[5] Margoliash, E., in Shemin, D., ed., *Biochemical Preparations*, **5**, 33 (1957).
[6] Hill, R. L., and Schmidt, W. R., *J. Biol. Chem.* (in the press).
[7] Spackman, D. H., Stein, W. H., and Moore, S., *Anal. Chem.*, **30**, 1190 (1958).
[8] Ingram, V. M., *Biochim. Biophys. Acta*, **28**, 539 (1958).
[9] Sanger, F., *Biochem. J.*, **39**, 507 (1945).
[10] Edman, P., *Acta Chem. Scand.*, **4**, 283 (1950); **7**, 700 (1953).
[11] Hill, R. L., Spackman, D. H., Brown, D. M., and Smith, E. L., in Vestling, C. S., ed., *Biochemical Preparations*, **6**, 35 (1958).
[12] Harris, J. I., *Biochem. J.*, **71**, 451 (1959).
[13] Narita, K., *Biochim. Biophys. Acta*, **28**, 184 (1958).
[14] Marshall, R. D., and Neuberger, A., *Biochem. J.*, **78**, 31*P* (1961).
[15] Harris, J. I., and Hindley, J., *J. Mol. Biol.*, **3**, 117 (1961).
[16] Margoliash, E., *Nature*, **175**, 293 (1955).
[17] Matsubara, H., Hagihara, B., Horio, T., and Okunuki, K., *Nature*, **179**, 250 (1957).
[18] Titani, K., Ishikura, H., and Minakami, S., *J. Biochem.* (Tokyo), **44**, 499 (1957).

Peptides, Terminal and Internal, released by Digestion with Trypsin

According to recent analyses, horse heart cytochrome *c* contains 2 arginine (refs. 1–4) and 18 (refs. 1, 2, 4) or 19 (ref. 3 and preceding communication) lysine residues per molecule. Tryptic digestion was therefore expected to yield 21 or 22 fragments. However, since the commercial trypsin used (Worthington, twice cryst.) exhibited a slight chymotryptic activity, a greater number of peptides was obtained. In order to minimize chymotryptic activity, the enzyme was pre-treated with dilute hydrochloric acid[5] and the duration of the enzymatic digestion of cytochrome *c* was reduced to 2 hr. The peptide mixture obtained was resolved on sheets of Whatman paper 3 *MM* with the help of a two-dimensional 'finger-printing' technique very similar to that introduced by Ingram[6]. The peptides were located with the help of ninhydrin or by observing the fluorescence which appeared on heating the sheets at 105° for 10 min. (ref. 7). The resulting peptide pattern is shown in Fig. 1.

For the qualitative determination of their amino-acid composition, the peptides from one sheet were eluted, hydrolysed with 5·7 *N* hydrochloric acid (containing 1 per cent thioglycollic acid in the case of peptides containing methionine), and the hydrolysates were analysed by paper chromatography (Table 1). When larger amounts of peptide were required for end-group determination by the dinitrophenyl method and for sequential analysis, the material eluted from several 'fingerprints' was combined. Alternatively, a larger-scale resolution of the peptide mixture obtained on tryptic digestion was carried out in two consecutive steps: 8-mgm. portions of the digest were first subjected to one-dimensional paper electrophoresis so as to give a series of fractions of different mobility; corresponding fractions eluted from several electrophoretic runs were then combined and purified by chromatography on Whatman 3 *MM* paper.

The methods used for elucidating the amino-acid sequence in most of the peptides occurring in the tryptic digest of horse cytochrome *c* (Table 1) were, largely, the classical ones introduced by Sanger, such as end-group determinations and partial hydrolysis by acid and by various enzymes, followed by characterization of the resulting smaller fragments.

Special attention was given to the peptides occupying *N*- and *C*-terminal positions in the polypeptide chain of cytochrome *c*. The search was stimulated by the fact that numerous attempts to identify an amino-acid carrying a free α-amino group had been unsuccessful (Paléus, S., and Tuppy, H., unpublished work). Earlier reports claiming a histidino[8] or an arginine residue[9] to be *N*-terminal could not be confirmed.

In the tryptic digest of horse heart cytochrome *c*, a peptide (*T*-20) was observed which gave a very weak ninhydrin colour and which was devoid of a free α-amino group. Cleavage by pepsin resulted in a tripeptide, Val-Glu-Lys, and an acidic fragment which failed to react with ninhydrin. The latter, on total acid hydrolysis, gave glycine and aspartic acid, whereas partial hydrolysis with 2 *N* hydrochloric acid (40 min., 100°) yielded the dipeptide Gly.Asp; heating at *p*H 2 (90 min., 105°) produced free aspartic acid and a substance chromatographically indistinguishable from acetylglycine; hydrazinolysis gave aspartic acid and two hydrazides behaving like glycyl-

Table 1. Peptides from Tryptic Hydrolysates of Horse Cytochrome *c*

	Amino-acids in the hydrolysate *	Proposed structure †	Remarks
T-20	Asp,Glu,Gly,Lys,Val	Acetyl-Gly-Asp-Val-Glu-Lys	*a*
T-2	*Gly*,Lys	Gly-Lys-Lys	*b*
T-4	*Gly*,Lys	Gly-Lys	
T-12	Glu,*Ileu*,Lys,Phe,Val	Ileu-Phe-Val-GluN-Lys	*c*
Hæm peptide	Ala,*Cys*,Cys,Glu,His,Lys,Thr,Val	Cys-Ala-GluN-Cys-His-Thr-Val-Glu-Lys (18)	
T-6*a*	*Gly*,Gly,Lys	Gly-Gly-Lys	*d*
T-2*a*	*His*,Lys	His-Lys	*b*
T-10*a*	Arg,Asp,Gly,His,Leu,Phe,Pro,*Thr*	Thr-Gly-Pro-AspN-Leu-His-Gly-Leu-Phe-Gly-Arg	*e*
T-17	Ala,Asp,Glu,Gly,Lys,Phe,Pro,*Thr*,Thr,Tyr	Thr-(Ala,GluN,Gly,Pro)-Phe-Thr-Tyr-Thr-(Ala,Asp)-Lys	*f*
T-6*b*	*Asp*,Lys	AspN-Lys	*d*
T-11*a*	*Gly*,Ileu,Lys,Thr,Try	Gly-Ileu-Thr-Try-Lys	*g*
T-19	Asp,*Glu*,Glu,Leu,Lys,Met,Pro,Thr,Tyr	Glu-(Glu,Thr,Leu)-(Glu,Met)-Tyr-Leu-Glu-AspN-Pro-Lys	*h*
T-11	Gly,Ileu,Lys,Pro,Thr,*Tyr*	Tyr-Ileu-(Pro,Gly)-Thr-Lys	*i*
T-12*b*	Ala,Gly,Ileu,Lys,*Met*,Phe	Met-Ileu-Phe-Ala-Gly-Ileu-Lys	*k*
T-1	*Lys*,Lys	Lys-Lys	
T-8*a*	Arg,Glu,*Lys*,Lys,Thr	Lys-Lys-Thr-Glu-Arg	*l*
T-8	Arg,Glu,*Lys*,Thr	Lys-Thr-Glu-Arg	
T-15	Arg,Glu,*Thr*	Thr-Glu-Arg	
T-19*a*	Ala,Asp,*Glu*,Ileu,Leu,Lys,Tyr	Glu-Asp-Leu-Ileu-Ala-Tyr-Leu-Lys	*m*
T-18	*Ala*,Asp,Glu,Thr	Ala-Thr-AspN-Glu	*n*

Peptides are designated by the prefix *T* to indicate their origin from a tryptic digest

* The *N*-terminal amino-acid determined by the dinitrophenol method is given in italics.

† Where amino-acids have been enclosed in brackets, neither their order nor molar ratio has been determined.

(*a*) Pepsin treatment gave acetyl-Gly-Asp and Val-Glu-Lys.

(*b*) The peptides *T*-2 and *T*-2*a* were separated by electrophoresis at *p*H 6·4

(*c*) Chymotrypsin, pepsin and subtilisin produced the same fragments (Ileu,Phe) and Val-GluN-Lys. On partial hydrolysis (Ileu,Phe,Val) was formed in good yield.

(*d*) The mixture of *T*-6*a* and *T*-6*b* could be resolved by electrophoresis at *p*H 2.

(*e*) Chymotrypsin split *T*-10*a* into two peptides: Gly-Arg (*T*-5) and (Thr,Gly,Pro,AspN,Leu,His)-Phe (*T*-12*a*). Degradation with subtilisin gave Thr-(Gly,Pro,AspN), Leu-His, Gly-Leu, (Gly,Leu,Phe), free Phe and Gly-Arg. *T*-12*a* was further degraded with pepsin yielding (Thr,Gly,Pro AspN,Leu) and (His,Gly,Leu,Phe). Partial hydrolysis of these two fragments gave (Thr,Gly), (Gly,Pro), (Gly,Pro,Asp,Leu), (Asp,Leu), (His,Gly) and (Leu,Phe).

(*f*) Chymotrypsin gave rise to Thr-(Ala,GluN,Gly,Pro)-Phe,Thr-Tyr and Thr-(Asp,Ala)-Lys. In a peptic hydrolysate of *T*-17 the presence of (Tyr,Thr,Ala,Asp)-Lys was established.

(*g*) Chymotrypsin split *T*-11*a* into Gly-(Ileu,Thr)-Try and free Lys. In a partial hydrolysate (Gly,Ileu) was found in addition to free amino-acids.

(*h*) Chymotryptic cleavage gave (Glu,Thr,Leu,Met)-Tyr and (Leu,Glu,AspN,Pro)-Lys (*T*-14). Peptic cleavage gave (Glu,Thr,Leu), (Met, Glu), (Met,Glu,Tyr,Leu and (Glu,AspN,Pro)-Lys. Partial hydrolysis of *T*-14 gave (Leu,Glu) and (Pro,Lys).

(*i*) Cleavage by subtilisin gave Tyr, (Ileu,Pro,Gly), (Ileu,Pro,Gly,Thr,Lys) and Thr-Lys. (Tyr,Gly,Pro,Ileu), (Ileu,Pro,Gly) and Thr-Lys were identified in partial hydrolysates. Tyr and Ileu were released from *T*-11 under the action of 'oxytocinase' (ref. 16).

(*k*) Degradation with chymotrypsin yielded (Met,Ileu)-Phe and Ala-(Gly,Ileu)-Lys. Partial hydrolysis of the latter gave (Ileu,Lys) and traces of (Gly,Ileu,Lys).

(*l*) Tryptic cleavage gave: Lys-Lys (*T*-1), Thr-Glu-Arg (*T*-15), free Lys and (Lys,Thr,Glu)-Arg (*T*-8).

(*m*) Chymotrypsin cleavage yielded (Ala,Glu,Asp,Ileu,Leu)-Tyr (*T*-21) and Leu-Lys (*T*-9). *T*-21 was further degraded with pepsin, the resulting two peptides being Glu-Asp-Leu and Ileu-Ala-Tyr.

(*n*) Glu is *C*-terminal (hydrazinolysis). Subtilisin cleavage gave Ala-Thr and AspN-Glu. Ala, (Thr,Asp,Glu) and (Thr,AspN,Glu) were found in partial hydrolysates.

hydrazide and acetyl-hydrazide. *T*-20 is, therefore, taken to be AcetylGly.Asp.Val.Glu.Lys and to represent the *N*-terminal portion of cytochrome *c*. Thus, cytochrome *c* can be added to the growing list of proteins and polypeptides characterized by an acetylated *N*-terminal amino-acid residue, namely, the proteins of tobacco mosaic[10], cucumber 4 (ref. 11) and turnip yellow mosaic virus[12], porcine α-melanocyte-stimulating hormone[13], and bovine fibrinogen[14].

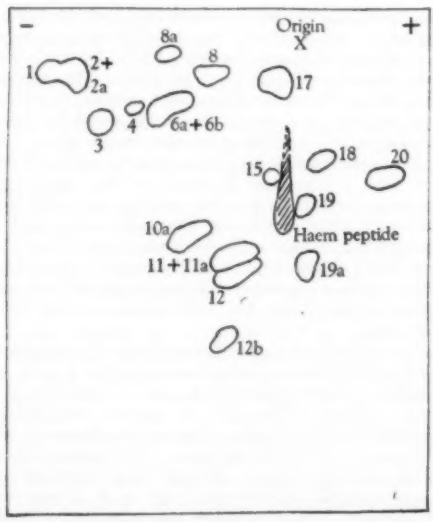


Fig. 1. 'Finger-print' of horse cytochrome *c*. 2 hr. digestion (37°, *p*H 8) of cytochrome *c* with trypsin (20 : 1, w/w) followed by paper electrophoresis (pyridine–acetate buffer, *p*H 4·7) and chromatography (*n*-butanol, acetic acid, water, 4 : 1 : 5, v/v/v). The trypsin had been preincubated with diluted hydrochloric acid

When trypsin virtually free of chymotryptic activity was used for the digestion of horse cytochrome *c*, all the fragments arising in good yield contained *C*-terminal lysine or arginine, except for one (*T*-18) the *C*-terminal residue of which, as determined by hydrazinolysis, proved to be glutamic acid. Glutamic acid had previously been reported by Titani *et al.*[15] to be in the *C*-terminal position in horse cytochrome *c*, a finding that we could readily confirm. This obviously suggests that *T*-18, the structure of which has been determined to be Ala.Thr.AspN.Glu, is derived from the *C*-terminus of the polypeptide chain of horse cytochrome *c*.

While this work was in progress, we learned from Prof. E. L. Smith that an investigation of the chymotryptic peptides of cytochrome *c* was going on in his laboratory. It is a pleasure to acknowledge the co-operative exchange of information which has led to simultaneous and joint publication of results. Full details of this work will be published in *Monatshefte für Chemie*. We wish to thank the Rockefeller Foundation for a grant.

G. Kreil
H. Tuppy

Institut für Biochemie,
University of Vienna.

[1] Nunnikhoven, R., *Biochim. Biophys. Acta*, **28**, 108 (1958).
[2] Leaf, G., Gillies, N. E, and Pirrie, R., *Biochem. J.*, **69**, 605 (1958).
[3] Holleman, J. W., and Biserte, G., *Bull. Soc. Chim. Biol.*, **41**, 975 (1959).
[4] Margoliash, E., *Fed. Proc.*, **19**, 217 (1960).
[5] Redfield, R. P., and Anfinsen, C. B., *J. Biol. Chem.*, **221**, 385 (1956).

[9] Ingram, V. M., *Biochim. Biophys. Acta*, **28**, 539 (1958).
[10] Phillips, D. M. P., *Nature*, **161**, 53 (1948).
[11] Margoliash, E., *Nature*, **175**, 293 (1955).
[12] Matsubara, H., Hagihara, B., Horio, T., and Okunuki, K., *Nature*, **179**, 250 (1957).
[13] Narita, K., *Biochim. Biophys. Acta*, **28**, 184 (1958).
[14] Narita, K., *Biochim. Biophys. Acta*, **31**, 372 (1959).
[15] Harris, J. I., and Hindley, A., *J. Mol. Biol.*, **3**, 117 (1961).
[16] Harris, J. I., and Ross, P., *Biochem. J.*, **71**, 434 (1959).
[17] Folk, J. E., and Gladner, J. A., *Biochim. Biophys. Acta*, **44**, 383 (1960).
[18] Titani, K., Ishikura, H., and Minakami, S., *J. Biochem.* (Tokyo), **44**, 499 (1957).
[19] Tuppy, H., and Wintersberger, E., *Monatsh.*, **91**, 1001 (1960).
[20] Tuppy, H., and Bodo, G., *Monatsh.*, **85**, 1024 (1954).

The Complete Amino-acid Sequence

FROM the work reported in the two preceding communications, it became possible to derive a unique amino-acid sequence for horse heart cytochrome *c*. This sequence of 104 amino-acid residues, together with the major points of hydrolysis by chymotrypsin and trypsin, is given in Fig. 1.

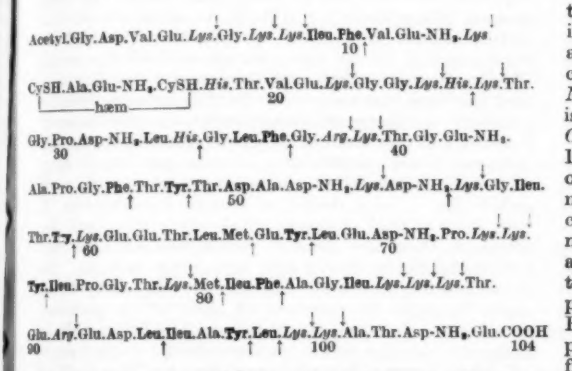


Fig. 1. Amino-acid sequences of horse heart cytochrome *c*. The arrows above and below the line indicate the points of hydrolysis by trypsin and chymotrypsin, respectively. The basic residues are in italics; the hydrophobic residues are in bold-faced type

In the following discussion the numbers used to identify the peptides recovered from cytochrome *c* after digestion with chymotrypsin and trypsin will be preceded by the letters *C* and *T*, respectively. The assignment of the sequence, as shown in Fig. 1, is based on the 13 largest peptides obtained from the chymotryptic digest[1] and the 20 peptides separated from the tryptic digest[2] as follows.

Peptides *C*-XIX*a* and *T*-20 both contain the *N*-terminal sequence of the protein, since the *N*-terminal α-amino group of both these peptides is acetylated. Peptides *T*-20, *T*-2 and *T*-4 are portions of the amino-acid sequence of *C*-XIX*a*, whereas *T*-12, Ileu.Phe.Val.Glu-NH_2.Lys, contains the *C*-terminal two residues of *C*-XIX*a*, Ileu.Phe and three additional residues. Indeed, the only other peptide from the chymotryptic digest containing an Ileu.Phe sequence is the dipeptide *C*-XIV*a*, obtained in 53 per cent yield; if *C*-XIV*a* were to represent the *N*-terminal portion of *T*-12 it would have to be preceded by a residue which affords a bond highly sensitive to both chymotrypsin and trypsin, which is an unlikely possibility. Moreover, *C*-XIV*a* was afterwards located in the interior of *T*-12*b* in the sequence Met.Ileu.Phe, which explains the release of Ileu.Phe in good yield by chymotrypsin.

The protein contains three valine residues, all of which are present in a Val.Glu.Lys sequence. Two of these sequences are in the hæm peptides obtained from chymotryptic and peptic digests (ref. 1 and preceding communication), whereas the third is in the *N*-terminal sequence present in *C*-XIX*a*. Since *T*-12 continues *C*-XIX*a*, the latter is necessarily followed by the hæm peptide resulting from digestion with chymotrypsin, which like the hæm peptide from peptic digests has the *N*-terminal sequence Val.Glu-NH_2.Lys. Of the three Val.Glu.Lys sequences, the *N*-terminal sequence of the hæm peptide is the only one which contains a glutaminyl rather than a glutamyl residue, further strengthening this conclusion. It should be noted that peptide *T*-6*a* with the unique sequence Gly.Gly.Lys is part of the *C*-terminal portion of the chymotryptic hæm peptide. The above argument thus establishes the sequence of the protein from the *N*-terminal end to residue 26.

The dipeptide *T*-2*a*, His.Lys, contains the histidyl residue *C*-terminal in the chymotryptic hæm peptide, since of the three histidyl residues in the protein the other two are in the interior of the hæm peptide in a His.Thr. sequence and in *C*-XXV*a* or *T*-10*a* in a His.Gly sequence. The peptide following the chymotryptic hæm peptide must, therefore, have an *N*-terminal lysyl residue. Three such peptides were isolated from the chymotryptic digest, *C*-XXV*a*, *C*-XVIII*b* and *C*-XXIII*a*. Peptide *C*-XXIII*a*, Lys.Lys.Ala.Thr.Asp-NH_2.Glu, is the only peptide obtained from the chymotryptic digest that does not have a *C*-terminal residue at which chymotrypsin could hydrolyse a peptide bond and for which it was not possible to show that it is followed in the overall amino-acid sequence by another peptide. It was therefore assigned to the *C*-terminal position in the protein (preceding communication by E. M. and E. L. S.). Similarly, the corresponding *C*-terminal peptide *T*-18, Ala.Thr.Asp-NH_2.Glu, was obtained from the tryptic digest (preceding communication by G. K. and H. T.). *C*-XXIII*a* can therefore be eliminated as a possible candidate for continuation of the chymotryptic hæm peptide. On the other hand, from the *C*-terminal end of the protein, peptide *C*-XVIII*b* follows the single tryptophan-containing peptide, *C*-XX*a*, because of the series of overlapping unique sections of chymotryptic and tryptic peptides discussed later and thus is located in the second half of the overall amino-acid sequence. Hence, *C*-XXV*a*, Lys.Thr.Gly.Pro.Asp-NH_2.Leu.His.Gly.Leu.Phe, is the only peptide in the chymotryptic digest which can represent the sequence immediately following the chymotryptic hæm peptide. This conclusion is supported by the finding that, from a partial chymotryptic digest, a hæm peptide was isolated which contains, in addition to the minimal chymotryptic hæm peptide listed in Table 1 of the preceding communication (by E. M. and E. L. S.), also the amino-acid sequence of *C*-XXV*a*.

The entire amino-acid sequence of *C*-XXV*a*, except for the *N*-terminal lysyl residue, is present in *T*-10*a*, which also contains a Gly.Arg. sequence at its *C*-terminal end. This Gly.Arg sequence is unique in the entire protein and *C*-XXVI*a* possesses Gly.Arg at its *N*-terminal end. Thus, *T*-10*a* provides a definitive overlap for placing *C*-XXVI*a* immediately after *C*-XXV*a*.

The unique composition and the *N*-terminal residue of the first half of peptide *T*-17, Thr(Ala,Glu-NH_2,Gly,Pro)Phe.Thr.Tyr.Thr.Asp.(Ala,Asp)Lys shows

that the *N*-terminal portion of this peptide contains the same sequence as the *C*-terminal part of *C*-XXVI*a*, the complete sequence of which is Gly.Arg.Lys.Thr.-Gly.Glu-NH_2.Ala.Pro.Gly.Phe. Moreover, the *C*-terminal portion of the sequence of *T*-17 clearly contains the dipeptide *C*-XIII*a*, Thr.Tyr, as well as the first five residues of *C*-XX*a*, Thr.Asp.Ala.Asp-NH_2.Lys.Asp-NH_2.Lys.Gly.Ileu.Thr.Try. Hence the derived sequence of chymotryptic peptides is *C*-XXV*a*.*C*-XXVI*a*.*C*-XIII*a*.*C*-XX*a*, and the sequence of tryptic peptides is *T*-2.*T*-10*a*.*T*-3.*T*-17, extending the overall amino-acid sequence to the 59th residue.

Peptide *T*-11*a* includes the *C*-terminal sequence of *C*-XX*a*, as well as a *C*-terminal lysine residue, thus showing that the chymotryptic peptide following *C*-XX*a* is one of the three having an *N*-terminal lysyl residue (*C*-XXV*a*, *C*-XVIII*b* or *C*-XXIII*a*). The same argument applies in this case as for the demonstration of the adjacent positions of the hæm peptide and *C*-XXV*a* already discussed above. Peptide *C*-XXIII*a* cannot follow *C*-XX*a* as it represents the *C*-terminal sequence of the protein. Since the remaining part of the amino-acid sequence of the protein can readily be established from the *C*-terminal end of the overall sequence to peptide *C*-XVIII*b*, *C*-XVIII*b* is the only possible continuation of *C*-XX*a*.

The *N*-terminal half of peptide *T*-19, Glu(Glu,Leu,-Thr)(Glu,Met) Tyr.Leu.Glu.Asp-NH_2.Pro.Lys, clearly contains the same sequence as *C*-XVIII*b*, Lys.Glu.-Glu.Thr.Leu.Met.Glu.Tyr, except for the *N*-terminal lysyl residue. On the other hand, the *C*-terminal portion of *T*-19 contains the sequence of the first five residues of *C*-XXIV*a*, Leu.Glu.Asp-NH_2.Pro.-Lys.Lys.Tyr, showing that *C*-XXIV*a* immediately follows *C*-XVIII*b*.

Since the *C*-terminal sequence of *C*-XXIV*a* is Lys.Lys.Tyr., the following tryptic peptide must be one having an *N*-terminal tyrosyl residue. Only one such tryptic peptide was found, namely, *T*-11, with the sequence Tyr.Ileu(Pro,Gly)Thr.Lys. The other three tyrosyl residues in the protein are present in the interior of tryptic peptides *T*-17, *T*-19 and *T*-19*a*, which contain the *C*-terminal tyrosyl residues of *C*-XII*a*, *C*-XVIII*b* and *C*-XXVIII*a*, respectively. The *C*-terminal sequence of *T*-11 is unique and corresponds to the first five residues of *C*-XVIII*a*, Ileu.Pro.Gly.Thr.Lys.Met. Thus, *C*-XVIII*a* follows *C*-XXIV*a* and the overall sequence is established to the 80th residue.

The *C*-terminal sequence of *C*-XVIII*a* is Lys.Met., hence the following tryptic peptide must contain an *N*-terminal methionyl residue. The only such peptide is *T*-12*b*, Met.Ileu.Phe.Ala.Gly.Ileu.Lys. The only other methionyl residue in the protein is present in the interior of *C*-XVIII*b* and *T*-19. Peptide *T*-12*b* contains, in addition to the second methionine in the protein, the dipeptide *C*-XIV*a*, Ileu.Phe, and the first four residues of *C*-XXVIII*a*. This unique sequence, Ala.Gly.Ileu.Lys, constitutes the four *C*-terminal residues of *T*-12*b* and the four *N*-terminal residues of the fifteen-residue chymotryptic peptide *C*-XXVIII*a*, Ala.Gly.Ileu.Lys.Lys.Lys.Thr.Gly.Arg.-Glu.Asp.Leu.Ileu.Ala.Tyr; this demonstrates that *C*-XXVIII*a* follows *C*-XIV*a*, which itself follows *C*-XVIII*a*. It should be noted that *C*-XXVIII*a* contains the sequences of the tryptic peptides *T*-8, *T*-8*a* and *T*-19*a*, so that the correct sequence of the tryptic peptides is *T*11.*T*-12*b*.*T*-8*a*.T-19*a*.

The six residues at the *N*-terminal portion of the octapeptide *T*-19*a*, Glu.Asp.Leu.Ileu.Ala.Tyr.Leu.-Lys., are in the same sequence as the six residues at the *C*-terminal part of peptide *C*-XXVIII*a*, showing that the last two residues of *T*-19*a*, Leu.Lys, follow the *C*-terminal tyrosyl residue of *C*-XXVIII*a*, thereby placing leucine (*C*-IV*a*), the only free amino-acid recovered in the chymotryptic digest. The chymotryptic peptide following *C*-IV*a* must contain *N*-terminal lysine and is, therefore, *C*-XXIII*a*, Lys.Lys.Ala.Thr.Asp-NH_2.Glu, the only chymotryptic peptide with *N*-terminal lysine not yet allocated and which represents the *C*-terminal sequence of the protein. It should be recalled (preceding communications) that cytochrome *c* possesses a *C*-terminal glutamic acid residue. Such a residue would not be expected to be *C*-terminal in peptides derived from chymotryptic or tryptic digests.

Discussion

The amino-acid sequence of horse heart cytochrome *c* given in Fig. 1 accounts for the entire composition and amide distribution of the protein. From this sequence the calculated molecular weight of the protein (Margoliash, E., Hill, R. L., Kimmel, J. R., and Schmidt, W. R., in preparation) is 12,398, corresponding to an iron content of 0·45 per cent. These values agree well with those previously determined from the iron content of the chromatographically purified[2] or the crystalline protein[3], on the basis of a content of one atom of iron per mole of protein.

Horse heart cytochrome *c* does not show any obvious relationship in its amino-acid sequence to either the α- or β-chains of human hæmoglobin *A* (ref. 4) or to that of sperm whale myoglobin (ref. 5), although there are distinct similarities between the two hæmoglobin chains and myoglobin (ref. 6). In horse heart cytochrome *c* the hæm is bonded by thiol ether linkages to residues 14 and 17, a position which is much nearer the *N*-terminal end of the chain than the location of the non-covalently bound hæm of either hæmoglobin or myoglobin[7,8]. This lack of similarity is not unexpected in view of the different functions of cytochrome *c* and the oxygen-carrying hæm proteins.

Some noteworthy features of the amino-acid sequence of cytochrome *c* are as follows:

(1) The *N*-terminal α-amino group is acetylated, a situation which has also been shown to occur in several other cases[9], already cited in the preceding communications. Acylated *N*-terminal residues may be expected to occur in other situations, particularly if one considers that *N*-terminal amino-acids have not been found in a number of other proteins by chemical methods.

(2) Those acidic residues which are not present as amides appear to have some tendency to be found in groups. Thus, there are Asp.Val.Glu, Glu.Glu.-Glu.Tyr.Leu.Glu. and Glu.Arg.Glu.Asp sequences. However, two glutamyl and one aspartyl residues are not in these areas.

(3) On the other hand, the basic amino-acids of cytochrome *c* are mainly grouped in small regions of the protein chain. These residues are shown in italics in Fig. 1. Of the nineteen lysyl, two arginyl and three histidyl residues, there are Lys.Gly.Lys.Lys, Lys.Gly.-Gly.Lys.His.Lys, Arg.Lys, Lys.Asp-NH_2.Lys, Lys.-Lys.Lys and two Lys.Lys sequences. Only three lysyl, one arginyl and two histidyl residues are not included in these sequences.

(4) There is an even more remarkable grouping of large hydrophobic residues (Leu.Ileu,Phe,Tyr,Try)

which are marked in bold-faced letters in Fig. 1. The chain includes the following sequences of such residues: Ileu.Phe, Leu.His.Gly.Leu.Phe, Phe.Thr.Tyr, Ileu.Thr.Try, Leu.Met, Glu.Tyr.Leu, Tyr.Ileu, Ileu.Phe.Ala.Gly.Ileu, and Leu.Ileu.Ala.-Tyr.Leu.

Cytochromes *c* of the mammalian type, defined as those cytochromes *c* that can readily interact with the appropriate enzyme systems of the terminal oxidation chain obtained from mammalian sources, have been isolated from a large variety of organisms. These include mammals, birds, fish and plant cells. At least twelve of these cytochromes *c* have been crystallized to date. All have molecular weights of about 12,000, a basic isoelectric point and an oxidation reduction potential of about + 0·250 V. It will be of interest to ascertain whether this similarity of function and physical properties is expressed in amino-acid sequences that show the same striking features as the protein from horse heart. A comparison of the amino-acid sequences of cytochromes *c* of the mammalian type should yield a picture of those portions of the pattern which are essential for the formation of the specific native three-dimensional structure that constitutes cytochrome *c*, and of those parts of the amino-acid sequence which can vary within the limits set by the structural requirements of the molecule. Thus far, such a comparison has been limited to a study of sequences adjoining the hæm group in certain cytochromes[10].

We wish to acknowledge the aid furnished by grants from the National Institutes of Health, U.S. Public Health Service (E. L. S.) and the Rockefeller Foundation (H. T.).

E. Margoliash*
Emil L. Smith
Laboratory for the Study of Hereditary and Metabolic Disorders,
and the
Departments of Biological Chemistry and Medicine,
University of Utah College of Medicine,
Salt Lake City, Utah.

Gunther Kreil
Hans Tuppy
Institut für Biochemie,
University of Vienna.

* Present address: McGill-Montreal General Hospital Research Institute, Montreal.

[1] Tuppy, H., and Paléus, S., *Acta Chem. Scand.*, **9**, 353 (1955).
[2] Margoliash, E., *Biochem. J.*, **56**, 535 (1954).
[3] Bodo, G., *Nature*, **176**, 829 (1955).
[4] Braunitzer, G., Rudloff, V., Hilse, K., Liebold, B., and Müller, R., *Z. physiol. Chem.*, **320**, 283 (1960). Braunitzer, G., Hilschmann, N., Hilse, K. Liebold, B., and Müller, R., *ibid.*, **322**, 96 (1960). Hill, R. J., and Konigsberg, W., *J. Biol. Chem.*, **236**, *PC7* (1961).
[5] Edmundson, A. B., and Hirs, C. H. W., *Nature*, **190**, 663 (1961). Kendrew, J. C., Watson, H. C., Strandberg, B. E., Dickerson, R. E., Phillips, D. C., and Shore, V. C., *ibid.*, **190**, 666 (1961).
[6] Watson, H. C., and Kendrew, J. C., *Nature*, **190**, 670 (1961).
[7] Perutz, M. F., Rossmann, M. G., Cullis, A. F., Muirhead, H., Will, G., and North, A. C. T., *Nature*, **185**, 416 (1960).
[8] Kendrew, J. C., Dickerson, R. E., Strandberg, B. E., Hart, R. G. and Davies, D. R., *Nature*, **185**, 422 (1960).
[9] Harris, J. I., *Biochem. J.*, **71**, 451 (1959). Narita, K., *Biochim. Biophys. Acta*, **28**, 184 (1958). Marshall, R. D., and Neuberger, A., *Biochem. J.*, **78**, 31*P* (1961). Harris, J. I., and Hindley, J., *J. Mol. Biol*, **3**, 117 (1961).
[10] Paléus, S., and Tuppy, H., *Acta Chem. Scand.*, **13**, 641 (1959).

OBITUARIES

Prof. M. W. Travers, F.R.S.

Morris William Travers, emeritus professor of chemistry in the University of Bristol and the senior Fellow (by date of election) of the Royal Society, died on August 25 at the age of eighty-nine.

Travers was born into a medical family on January 24, 1872. He was educated at Blundell's School, Tiverton, and University College, London. After a period at the University of Nancy, he returned to University College as a demonstrator in 1894, and became assistant professor in 1898. The ten years from 1894 to 1904 were among the most fruitful in Travers's career, for he returned to London in time to play an active part in Ramsay's brilliant work which led to the discovery and examination of the rare gases. The excitement of this period lived vividly with Travers during the next sixty years, and he never tired of recalling the steps which led to these discoveries.

In 1904, shortly after his election to the Royal Society, Travers was appointed to the chair of chemistry at University College, Bristol. He brought with him the hydrogen liquefier which he had just perfected at London, and during his time at Bristol his research was concerned largely with low temperatures. His main interests, however, centred on the movement for the foundation of a University of Bristol. He wrote a pamphlet on the need for a University and the principles to be observed in its constitution; this pamphlet had wide circulation, and Travers enlisted both local and national support for his proposals. His enthusiasm was in no small measure responsible for the movement which culminated in the offer by H. O. Wills in 1908 of £100,000 towards the endowment of a University, but by this time Travers had left Bristol.

In 1907 he became director of the Indian Institute of Science at Bangalore, a post which he held until his return to the United Kingdom in 1914. On the outbreak of war it was soon realized that Great Britain would suffer a serious shortage of chemical glassware, which until then had all been imported from the Continent. Travers set to work to devise suitable glasses for chemical use and took his processes to a small glassworks in Walthamstow. The enthusiasm with which he had carried through his researches in the laboratory was equally effective in the factory, and very soon 'Duroglass' was in successful production.

After the First World War, Travers was concerned with furnace technology; he patented a furnace and for a while ran a factory to produce it. In 1922 he was elected president of the Society of Glass Technology. He returned to Bristol in 1927 as honorary professor, Fellow and Nash lecturer in chemistry. He now resumed academic research and staged a comeback on a topic new to him—the study of gaseous and heterogeneous reactions. In this field he brought to bear his superb experimental skill, and was ahead of his time in insisting on a complete and careful analysis of all the products of a reaction. His return to academic life culminated in his presidency of the Faraday Society in 1936. He did not, however, sever all links with industry; he became a consultant to the National Smelting Co., Ltd., in 1931, and continued to work in this capacity, apart from a break during 1940–45, either on a full- or part-time basis up to his death. In 1937 he retired from the Univer-

XUM

sity of Bristol and was accorded the title of emeritus professor.

At the start of the Second World War, Travers offered his services to the Royal Ordnance Factories, and, as a consultant, threw himself whole-heartedly into the diverse problems which had to be solved. He made many contributions, particularly in connexion with the application of silicon iron in sulphuric acid manufacture, and in the development of rocket cordite. In his dealings with technological problems he had a remarkable facility for putting his finger on the source of trouble. It was amazing to see a man, who was for most of the War more than seventy years of age, travel many miles, usually at night, to face the problems in the widely scattered ordnance factories. In this period he was a wonderful example to the younger scientists and technologists in his untiring enthusiasm for work.

Soon after the end of the War, Travers moved to Stroud and began what was for him one of his greatest and most congenial tasks, the production of a full biography of Sir William Ramsay. This work, which Travers had began in 1937, was taken up again in 1948, and the book was written between Travers's eighty-first and eighty-third birthdays; it is one of the great biographies of our time and it will surely remain as a monument to Sir William Ramsay and a fitting memorial to Travers.

Prof. Travers is survived by his wife, formerly Miss Dorothy Gray, a pianist of outstanding ability, whom he married in 1909, by their daughter and by their son, who is now professor of educational psychology at the University of Utah.

I am indebted to Mr. S. W. G. Morgan, Dr. W. G. Hiscock and Mr. J. Lumsden, of the Imperial Smelting Corporation, for providing information about Travers's industrial career.

D. H. Everett

Prof. R. H. Dastur, O.B.E.

India has lost a distinguished plant physiologist and agronomist by the death of Prof. R. H. Dastur, which occurred at Bombay on October 1, 1961, at the age of sixty-five years.

Rustom Hormusji Dastur was born on March 7, 1896. After graduating as bachelor of science in 1918 at the Gujarat College, Ahmedabad, he was appointed a demonstrator in botany at the same College and obtained the M.Sc. degree in botany, by research, in 1921. His association at the Gujarat College with the noted botanist Prof. W. T. Saxton gave young Dastur a good grounding in systematic botany and ecology. Dastur's interests, however, turned very early in his career towards plant physiology, despite the fact that he had little regular training in plant physiological research, except perhaps for a year (1924–25) he spent at the University of Reading, England, with Prof. W. Stiles. There he experimentally tested his earlier ideas on the relation of water content to photosynthesis (*Ann. Bot.*, **38**, 779; 1924) and made a new departure by measuring photosynthesis in a leaf attached to the plant (*Ann. Bot.*, **39**, 769; 1925).

Dastur was appointed assistant lecturer in botany at the Royal Institute of Science, Bombay, in 1921, and continued there as professor and head of the Department of Botany during 1929–1935. During this period of about half a decade he established a very active school of plant physiology, and his investigations, in collaboration with his pupils, related to the influence on photosynthesis of factors such as water and chlorophyll content, and quality and polarization of light, physiology and anatomy of tendrils, respiration, and physiology of the rice plant. The results of these investigations were published in the *Annals of Botany*, the *Journal of the Indian Botanical Society* and the *Indian Journal of Agricultural Science*.

In the year 1935 Dastur's interest shifted from the academic to the applied side of plant physiology, and work on the cotton crop claimed his entire interest for the next 26 years and constituted the *magnum opus* of his scientific output.

In 1919 the crop of the Panjab-American cotton, hitherto the main standby of the cultivator in the canal colonies of the Panjab, suffered a serious setback when it was attacked by a disease called 'tirak'. By 1935, another seven failures of the crop, partial or complete, occurred. Various theories were advanced to explain this condition but none satisfactorily accounted for and solved the mystery of the occasional failures of the crop. Prof. Dastur was then invited by the Indian Central Cotton Committee to undertake investigation into this problem, and it was indeed a far-sighted and bold step on the part of the Committee and Mr. H. R. (later Sir Herbert) Stewart, the then director of agriculture, Panjab, to pin their faith on one who until he undertook the investigation had no previous contact with or knowledge of the cotton plant. This investigation, carried out from 1935 until 1943 and described in several scientific papers and eventually in a monograph, was reviewed in *Nature* (**158**, 524; 1946). Sir Herbert Stewart summed it up in the words: "The story which it unfolds is a striking example of how investigations into the realm of pure science can be turned to practical account in everyday agricultural practice".

Dastur retired from service in 1951 on attaining the age of fifty-five years. The Indian Central Cotton Committee, however, retained his services in an honorary capacity until the time of his death for directing and supervising plant physiological and agronomic research schemes on cotton.

During 1944–61, Prof. Dastur was engaged in investigations on: (1) causes of bad opening of bolls in Sind; (2) causes of the low yield of sea-island and Egyptian cottons in the various cotton-growing tracts: (3) causes of red-leaf disease and several other related aspects, and obtained a number of important and useful results. These results were compiled in two other monographs which were published by the Indian Central Cotton Committee in 1959 and 1960. Prof. Dastur was in the process of constructing a theory for the application of fertilizers on the basis of a balance between nitrogen, phosphorus and potassium contents of the plant, and it is indeed unfortunate that death claimed him before he could complete this interesting and useful concept.

Dastur had a reserved, but gentle and kindly disposition and shunned publicity; he virtually lived for his work and had little interest in social activities and scientific conferences which would take him away from it. For his work on cotton in the Panjab he was made O.B.E. in 1946. He was a Foundation Fellow of the National Institute of Sciences of India and was elected president of the Botany Section of the Indian Science Congress in 1933 and of the Indian Society for Plant Physiology in 1959.

He leaves behind a widow, a daughter, a son and a large circle of pupils and colleagues who will cherish his memory for a long time to come.

R. D. Asana

NEWS and VIEWS

Post Office Radio Services Department : Mr. T. A. Davies, O.B.E.

Mr. T. A. Davies, inspector of wireless telegraphy in the Wireless Telegraph Section of the Radio Services Department of the Post Office, is to retire at the end of the year. Mr. Davies took a love of the sea to his job as head of the Wireless Telegraphy Section in 1948. This was to prove useful in his later work. After five years apprenticeship in the Royal Dockyard in his home city of Portsmouth, he obtained a first-class honours degree in engineering and joined the Post Office in 1925. While working in the Radio Branch of the Engineering Department he was able to use his knowledge of shipping in providing radio communications to ships and safety services for them while at sea. As inspector of wireless telegraphy he has been responsible for control and operation of the ship–shore services, inspection of ships' radio equipment, and the examinations for candidates for the Postmaster-General's Certificate. Twelve radio stations are administered from the headquarters in London.

Mr. R. Billington

Mr. Reg Billington has been appointed inspector of wireless telegraphy in the Wireless Telegraph Section of the Radio Services Department of the Post Office to succeed Mr. Davies. After obtaining an honours degree in the University of London, Mr. Billington worked in industry for two years before joining the Post Office Engineering Department in 1936. For a period during the Second World War he was seconded to the Iraq Government as chief engineer and returned to the Post Office in 1945, where he soon became an assistant inspector of wireless telegraphy. In 1948 he became deputy inspector.

Applied Electromagnetic Theory Section at the U.S. National Bureau of Standards : Mr. J. R. Johler

Mr. J. R. Johler has been appointed chief of the new Applied Electromagnetic Theory Section, National Bureau of Standards, U.S. Department of Commerce. The section will be located in the Radio Systems Division at the Laboratories of the National Bureau of Standards at Boulder, Colorado. Mr. Johler's Section will be responsible for developing techniques for the solution of electromagnetic theory problems in terrestrial radio-wave propagation by numerical analysis, analytic and asymptotic series techniques, and will direct its work to electronic computer solutions. Much of the Section's work will be devoted to programmes of other Sections and Divisions in providing theoretical computations of propagation in various media. Since 1953, Mr. Johler has worked principally in the field of radio navigation systems evaluation, in low and very low frequency radio-wave propagation, and sferics (radio noise caused by lightning flashes). During 1953–55 he was responsible for the theoretical prediction of the performance of certain pulse-type radio navigation systems. Before joining the National Bureau of Standards in 1946, Mr. Johler had worked on the computational and engineering details of exterior ballistics at Aberdeen Proving Ground, Aberdeen, Md.

Atlantic Regional Laboratory, National Research Council, Canada : Dr. A. C. Neish

Dr. A. C. Neish has been appointed director of the Atlantic Regional Laboratory of the National Research Council of Canada, at Halifax, Nova Scotia, in succession to Dr. E. Gordon Young. Dr. Neish studied at Nova Scotia Agricultural College; he obtained his B.Sc. (agriculture) from Macdonald College, majoring in agricultural chemistry, and his M.Sc. and Ph.D. (chemistry) from McGill University. His experience ranges from soils analysis and soils surveys at the Canada Department of Agriculture, and sessional lecturer, Department of Chemistry, McGill University (1942–43), to his research work with the National Research Council, first in the Division of Applied Biology (1943–48) and then with the Prairie Regional Laboratory at Saskatoon, Sask., where, in 1956, he was appointed head of the Physiology and Biochemistry of Plants Section.

Royal Society Expedition to Tristan da Cunha

The Royal Society is to send a scientific expedition to examine the recent volcanic activity on the island of Tristan da Cunha in the South Atlantic. This activity is of considerable interest to geologists in Great Britain and the proposal is to mount an expedition which will include four or five geologists, leaving Cape Town some time during January 1962, to spend 2–3 months on the island. It is also proposed to include a zoologist and a botanist in the expedition to study any effects which the evacuation of the population of the island may have had on the flora and fauna. The expedition will be concerned not only with a detailed examination of the present volcanic activity but also to determine as accurately as possible the past geological history of the Island. The scientific direction of the expedition is under the control of Prof. W. Q. Kennedy, professor of geology in the University of Leeds, with Prof. C. F. A. Pantin, professor of zoology in the University of Cambridge, as biological adviser, and with the co-operation of the Nature Conservancy.

H.M.S. *Jaguar* will be visiting Tristan da Cunha briefly later this month and advantage is being taken of this to send two geologists aboard the ship to make a preliminary investigation prior to the departure of the main expedition. These two geologists are Dr. R. W. Le Maitre, of the British Museum (Natural History), and Dr. P. G. Harris, of the Department of Geology, University of Leeds.

Science in Parliament

The Parliamentary Secretary for Science, Mr. D. Freeth, dealt with a series of questions on science on December 5, ranging from nuclear energy, red deer stags on the Island of Rhum, engineering designers, strontium research, radioactivity, medical research on animals, the emigration of scientists, space exploration, the employment of scientists and the report on the long-term demand for scientific manpower. Mr. Freeth said that the Minister for Science is not satisfied with the present supply of engineering

designers in Britain and he is in close touch with the President of the Board of Trade and trying to find ways to ameliorate the position. The Department of Scientific and Industrial Research at present employs 170 scientists in Scotland, and it is intended to increase this number, subject to assessments of changing priorities and the resources available. There are about 22,000 members of research associations, of whom a few belong to more than one association. British plans for space exploration do not include projects for sending men into space.

U.K. Expenditure on Science, Technology and Education

In a written answer in the House of Commons on November 28, the Parliamentary Secretary for Science, Mr. Denzil Freeth, said that the greater part of the expenditure on scientific and technological research in universities, estimated at £23·3 million in 1958–59 and £25·9 million in 1959–60, was met from the recurrent grants made by the Treasury on the recommendation of the University Grants Committee and was not separately identified. Research Council grants to universities and to research units and institutes associated with universities amounted to £3·1 million in 1957–58, £3·5 million in 1958–59, £4·5 million in 1959–60, £6 million in 1960–61 and £8·2 million in 1961–62. In figures circulated for the Research Councils, with the exception of the Overseas Research Council, the grants from the Department of Scientific and Industrial Research are given as £1,315,000 in studentships and fellowships and £2,600,000 in research grants for 1961–62, the corresponding figures for 1960–61 being £1,050,000 and £1,490,000. For the Agricultural Research Council in 1961–62, studentships and fellowships amounted to £31,000, research grants to £157,000, expenditure of research units within universities £273,000 and grants to research units or institutes associated with universities, £1,011,000; for the period 1960–61, the figures are £24,000, £104,000, £299,000, and £931,000. For the Medical Research Council the corresponding figures are £115,000, £811,000, £1,526,000, and £317,000 in 1961–62, and £100,000, £620,000, £1,060,000 and £285,000 in 1960–61. For the Nature Conservancy, they are £21,000, £20,000, £11,000 under the first three headings in 1961–62, and £18,000, £11,000 and £7,000 in 1960–61.

Replying to a question in the House of Commons on November 30, the Minister of Education, Sir David Eccles, said that the Ministry's present grant to the National Foundation for Educational Research was £7,000 and local education authorities were contributing £29,000 as members of the Foundation. The Ministry was at present in discussion with the Foundation, and Sir David undertook to see that the availability of information was included.

Toxic Chemicals in Agriculture

On December 5, the Joint Parliamentary Secretary to the Ministry of Agriculture, Fisheries and Food, Earl Waldegrave, announced publication of the report of the Research Study Group on Toxic Chemicals in Agriculture and Food Storage. He said that the report concluded that protective measures now in force are generally successful, but affirmed the need for more fundamental and applied research in this field. Earl Waldegrave said that the Government accepted the conclusions and intends to implement the Group's recommendations to the full extent of the additional resources which can be made available. The Minister, Mr. C. Soames, made a similar statement in a written answer in the House of Commons on the same day, but, when pressed, neither he nor Earl Waldegrave would add to that statement. In reply to further questions on December 7, Mr. Soames said that he would be replying next week to the Select Committee on Estimates, which, in its Sixth Report of June 28, had recommended an inquiry into the effect on agriculture, public health and the ecology of Britain of all chemicals used in agriculture, and that he hoped that the restrictions on the use of cereal seed dressings containing aldrin, dieldrin and heptachlor which he had announced on July 3 would prevent casualties in future among wild birds and mammals. His Department would intensify its survey of wild-life casualties as recommended by the Research Study Group, and consider after next session, with the interests concerned, whether any further action is necessary.

The Commonwealth Scholarship Commission in the United Kingdom

The Commonwealth Scholarship Commission was constituted in December 1959 under the Commonwealth Scholarships Act, and in accordance with the proposed arrangements for administering in the United Kingdom the Commonwealth Scholarship and Fellowship Plan, recommended by the Commonwealth Education Conference in July 1959 and set forth in the White Paper presented to Parliament. Pending the introduction of the relevant legislation and to facilitate the speedy implementation of the Plan, the Secretary of State for Commonwealth Relations had already appointed an interim committee, with Sir Eric Ashby as chairman, which met three times before the Commission held its first meeting on February 23, 1960, under the Earl of Scarborough as chairman, Sir Eric Ashby being then elected deputy chairman. Confirming the preliminary arrangements made by the interim committee, the Commission endorsed the view that in the first year attention should be concentrated on the selection of Commonwealth Scholars and invitation for senior Commonwealth Fellowships deferred for a year. According to the first annual report of the Commission, up to the end of April 1960, 452 applicants were nominated by overseas agencies from some 5,177 applications received, and from these a Selection Committee recommended 219 candidates to the Commission, of whom only 178 accepted the offer of scholarships (Cmnd. 1541. Pp. 26. London: H.M.S.O., 1961. 1*s.* 6*d.* net). Of these, 37 were from India, 17 each from Pakistan and Canada, 16 from Nigeria, 15 from Australia, 10 each from New Zealand and South Africa, and 8 from Hong Kong. Of these again, 74 were in the humanities, 50 in science, 33 in technology and 21 in medicine. The chief considerations in selection were intellectual merit and a realistic and practicable plan of study, but selections were also made so as to ensure that the various parts of the Commonwealth are appropriately represented. Applications have also been invited from candidates in the United Kingdom for awards offered by Canada, Australia, New Zealand, South Africa, Malaya, East Africa and Hong Kong, and scholarships have been offered by Canada to 13 candidates nominated.

Exchange of Students for Technical Experience

The fourteenth annual report of the International Association for the Exchange of Students for Technical Experience reports continued growth in 1961.

with three new members—Argentina, Luxembourg and the United Arab Republic—7,478 students exchanged, compared with 6,430 in 1960, and 3,265 employers offering training, an increase of 229. Since its foundation the Association has provided 59,769 students with technical training abroad. Exchanges of civil engineering and forestry students have increased considerably, but the exchange is still concentrated in a few European countries, and Germany, which received 1,757 students, with Great Britain and Sweden, represents more than 50 per cent of the training possibilities. Some 569 universities and colleges are now participating and 45 faculties are covered. These institutions and the participating firms are listed in the report by countries.

The corresponding report of the United Kingdom Association, which was established as an independent organization as from October 1, 1960, covers the year ending September 1961. During the summer vacation of 1961, 894 students were sent abroad for experience and 965 overseas students received and placed in British industry and commerce with the help of 389 British firms and organizations, compared with 883 and 908, respectively, in 1960. Of the students coming to Britain, Sweden (225), Germany (108), Netherlands (86), France (79) and Canada (57) sent the largest numbers, and of those received Sweden took 178, Germany 139 and France 117 (Fourteenth Annual Report, 1961. Pp. 79. Report of IAESTE in the United Kingdom for year ending September 1961. Pp. 14. London: International Association for the Exchange of Students for Technical Experience (U.K.), 1961).

History of Science and Technology at the Imperial College, London

Much thought is being given to-day, by those responsible for the education of scientists and technologists, to the best means of broadening their education and preventing excessive specialization. The Imperial College of Science and Technology is attacking this problem in various ways. A new development is now being added to those already in existence. A chair is being instituted in the history of science and technology which will result in a department for research and study of this subject in its own right; no exactly similar chair exists in a British university, although there are chairs elsewhere in the history and philosophy of science. The field of study, which is intentionally being made wide, will it is hoped embrace both the genesis of basic ideas and tools and the recent history of science and technology, the latter particularly because of the great acceleration in applying scientific discovery with important social and political results. The aim will be to teach scientists and engineers, rather than students of history, so that they become familiar with the background of major discoveries and inventions. To this end, the Department will start by providing postgraduate courses, both full-time and part-time. Later, it is hoped, optional undergraduate courses will be introduced in the third year of some degree courses. A generous contribution towards the cost of maintaining the new Department has been made by Imperial Chemical Industries, Ltd., which has recently supported the study of the history of technology by subsidizing some valuable publications. The establishment of the new Department will present both scientists and historians with a challenge; the first holder of the chair will have the opportunity to bridge one of the gaps between the humanities and the sciences. The results of this development will be watched with interest.

United Kingdom Fire Statistics, 1960

Since the Department of Scientific and Industrial Research and Fire Offices' Committee Joint Fire Research Organization began its statistical record in 1946, the number of fires in buildings occurring annually in the United Kingdom has almost doubled and, in 1960, was more than 62,000. The total number of fires of all kinds reached a peak in 1959 when, due to an exceptionally dry summer, a very large number of outbreaks occurred in the open. However, the figure for 1960, 133,484, is second only to that for 1959. These figures are revealed in *United Kingdom Fire Statistics 1960: Statistical Analysis of Report of Fires Attended by Fire Brigades in the United Kingdom during 1960*, which has recently been published as the first of an annual series made available to the general public (Pp. 106. London: H.M.S.O., 1961. 12*s.* 6*d.* net). This volume presents tables of figures relating to the origin and extinction of fires and to resultant casualties. Separate series are given for England and Wales, Scotland and Northern Ireland, and summary tables for the United Kingdom as a whole afford comparisons with previous years back to 1956. In the last-named section graphs are given of the annual and monthly incidence of fires, and there is a special graphical analysis of fires associated with space heating during the period 1949–60, this being a major cause of fire, particularly in dwellings. A marked downward trend in occurrences due to fires in open grates has been offset by a sharp rise in fires caused by oil-burning heaters, but the latter have been checked in the last year of the period under review. It is encouraging to note that the Fire Brigades are apparently relying increasingly on hose reel equipment for the first attack on fires and that this is proving adequate in a large proportion of cases. This should result in a continued reduction in water damage, although it is not possible to obtain figures to illustrate this point.

Dairy Research in Australia

In the small space of twelve short pages the Dairy Section of the Australian Commonwealth Scientific and Industrial Research Organization, led by Mr. G. Loftus Hills, presents a succinct account of its work for the year 1960–61 (Annual Report for the year ending June 30, 1961. Pp. 18. Melbourne: Commonwealth Scientific and Industrial Research Organization, Dairy Research Section, 1961). With a staff of about fifty people, of whom some twenty are graduates, the scope and value of the work now being done by the Section are very considerable. The topics under investigation include the mechanization of cheese-making, the surface deterioration of butter resulting from oxidative effects of the copper present in the parchment in which it is wrapped, and problems encountered in the manufacture of casein. The main buyers of Australian casein are paper manufacturers in the United States and Japan, where it is used for coating art paper, but this outlet for Australian casein is threatened by competition from soya bean proteins and from synthetic substances which give solutions of lower viscosity and which therefore allow the coating process to be done more rapidly than is at present possible with casein solutions. In an endeavour to solve this problem a detailed study is being made of the factors that affect the viscosity of

casein solutions. A series of investigations, extremely important not only in dairying but also to many other branches of the food industry, is that concerned with flavour chemistry. The object of this work is to determine what carbonyl and other compounds are responsible for the various off-flavours, such as oily, tallowy, fishy and cardboardy, that can occur in certain circumstances in dairy products, and, if possible, to isolate and characterize the substances concerned. Marked progress has already been made in that direction. The report ends with a list of twenty-nine papers published during the year, some in the British *Journal of Dairy Research*, but most of them in the *Australian Journal of Dairy Technology*. Judging from this impressive list of publications, the Dairy Section of the Commonwealth Scientific and Industrial Research Organization can certainly claim that it had a satisfactorily fruitful year in 1960–61.

Strangers' Hall, Norwich

During the past year, four rooms on the street front of Strangers' Hall have, with the help of a Carnegie Grant, been converted into period rooms. On the ground floor is a kitchen with furnishings of about 1830 and a Georgian sitting-room. These rooms are seen from the street, but visitors do not go into them. On the first floor is a Victorian night nursery of about 1880 and a Victorian sitting-room of about 1860. Visitors walk along a roped pathway through these rooms.

Field Studies Council : Courses for 1962

The programme of courses for 1962, organized under the auspices of the Field Studies Council, has recently been issued. Such courses vary from those suitable for pupils below sixth-form level to those for university students, teachers and amateurs. Most natural-history subjects are covered. They are held at the following Field Centres: Slapton Ley, Kingsbridge, Devonshire; Preston Montford, near Shrewsbury; Malham Tarn, near Settle, Yorkshire; Juniper Hall, Dorking, Surrey; Flatford Mill, East Bergholt, near Colchester. Further information can be obtained from the wardens of these Field Centres or from the Publicity Secretary, Field Studies Council, Ravensmead, Keston, Kent.

University News : Glasgow

The following have been appointed to lectureships: Adam Fleck (biochemistry); N. J. McCorkindale, I. C. McNeill, K. C. Campbell and A. L. Porte (chemistry); W. T. Morton (experimental physics); and D. L. Pursey (theoretical physics). A. J. Baker has been appointed Glaxo Fellow in chemistry; other fellowships have been awarded to: G. L. le Bouvier, June Dixon and K. R. Dronamraju (genetics); John Cumming, A. W. Hendry and J. G. Lynch (nuclear physics); Robert McCrindle, M. B. Meyers and R. D. H. Murray (organic chemistry); R. M. Turnbull (physics); and J. D. Roycroft (solid-state physics).

An Associated Electrical Industries (*RS2*) high-resolution nuclear magnetic resonance spectrometer (the cost of which was defrayed by the Department of Scientific and Industrial Research) is now in operation in the Chemistry Department.

For the present session, 392 students (292 men and 100 women) have been admitted to the science departments compared with 396 in 1960. The total of matriculated students in the University as a whole now stands at 6,765, compared with 6,600 in 1960.

London

Prof. Michael Abercrombie, professor of embryology in University College, has been appointed to the Jodrell chair of zoology and embryology at that College from October 1, 1962, in succession to Prof. P. B. Medawar (*Nature*, **187**, 991; 1960).

The following appointments to readerships have been announced: Dr. J. R. Butler (pure geochemistry) at the Imperial College of Science and Technology; Mr. J. C. G. Coleridge (physiology) at the Royal Free Hospital School of Medicine; Dr. S. J. Taylor (mathematics) at Westfield College; Mr. S. B. Watkins (chemical engineering) at King's College; Dr. F. W. O'Grady (bacteriology) at St. Bartholomew's Hospital Medical College; Dr. J. C. Anderson (electrical engineering) in the Imperial College of Science and Technology.

The title of professor has been conferred on: Mr. J. K. T. L. Nash (civil engineering), in respect of his post at King's College; Dr. H. E. Rose (powder technology), in respect of his post at King's College; Dr. J. H. Westcott (electrical engineering), in respect of his post at the Imperial College of Science and Technology. Titles of reader have been conferred on: Dr. K. W. Gruenberg (pure mathematics) in respect of his post at Queen Mary College; Dr. J. M. Zarek (engineering science) in respect of his post at King's College.

Announcements

The French Minister of Agriculture has conferred on Sir John Russell, formerly director of Rothamsted Experimental Station, the honour of Commander of the Order of Agricultural Merit on the occasion of the bicentenary of the French Academy of Agriculture.

Sir Charles Dodds, director of the Courtauld Institute of Biochemistry at the Middlesex Hospital Medical School, London, and a governor of the Lister Institute of Preventive Medicine since 1956, has been appointed chairman of the Governing Body of the Lister Institute, in succession to Sir Henry Dale, who has resigned after occupying that office for twenty years.

The Bradford Chemical Society and the Department of Chemical Technology of the Bradford Institute of Technology, in conjunction with the Yorkshire Council for Further Education, are organizing a symposium on "Antibiotics and Newer Synthetic Drugs", to be held at the Bradford Institute of Technology on February 10. Further information can be obtained from Dr. R. P. Sheldon, Department of Chemical Technology, Bradford Institute of Technology, Bradford.

The sixteenth annual symposium on fundamental cancer research, entitled "Conceptual Advances in Immunology and Oncology", is to be held in the University of Texas during March 1–3. Subjects under discussion will include theories of antibody production; control mechanisms of antibody synthesis; the nature of the antigen–antibody reaction; transplantation and immunological tolerance; and cancer specific antigens. Further information can be obtained from the Editorial Office, M. D. Anderson Hospital and Tumor Institute, University of Texas, Houston 25, Texas.

Erratum. In the communication entitled "Riometer Observations during a Solar Eclipse", by M. A. Gordon, in *Nature* of October 21, p. 249, the legend to Fig. 1 requires amendment: for "27·5 Mc./sec. radio wave" read "27·5 Mc./sec. (day) radio wave".

THE NEW MATHEMATICS AND OCEANOGRAPHY BUILDING OF THE UNIVERSITY OF LIVERPOOL

A FEW weeks ago the University of Liverpool took over from the building contractors (Messrs. Gilbert-Ash, Ltd.) an almost completed Mathematics and Oceanography Building, the architects of which are Bryan and Norman Westwood and Partners, of London. The building, which will cost about £260,000, has three wings, one on each of three sides of a grass-covered central courtyard; the remaining side of the courtyard will, at some later time, be open to one of the pathways of what will then be the University Precinct.

The main wing is a gracious six-storeyed structure of glass, black concrete pillars, and white concrete panels of special design. On the flat roof there is a 'wood and glass' penthouse in which the transcendental verities may be contemplated in relative comfort. Below the building is a basement for the usual services, and farther below still there is the area boiler house.

On the ground floor of the main wing, which is well set back from the road, and which is approached along a covered pathway traversing the courtyard, there is a spacious white-walled, airy, bright, central hall, the floor of which is made of attractive grey-green slate. On one of the walls of the hall there is a five-panel mural inspired by the users, designed by John McCarthy, and executed by him in red terossa ferrata. This mural represents, in various groups, the history and development of mathematics, and many of the important branches of human activity in which pure and applied mathematics play, or have played, a significant part. In addition, the hall contains a tall black-concrete abstract mural designed and executed by Eric Peskett. Leading off the central hall there will be, when completed, the two principal lecture rooms of the new building. The rooms will be large enough to hold audiences of 110 and 145, respectively, and they will be named after A. R. Forsyth and J. Proudman, the first professors of pure mathematics and applied mathematics of the University of Liverpool. Proudman was afterwards transferred to the University chair of oceanography, and did much to give the Department of that subject its high reputation; it is therefore a particularly happy arrangement whereby the Departments of Mathematics and Oceanography are now housed in the same building.

The first and second floors contain lecture rooms; the third floor contains more lecture rooms as well as the Sub-Department of Mathematical Statistics. The fourth floor contains private rooms for the applied mathematics staff, and the fifth floor has similar rooms for the pure mathematics staff, as well as rooms for research students.

At ground-floor level, at right-angles to the main block, there is a single-storey wing containing the Oceanography Laboratories; these are used for research and for instruction for honours under-

Fig. 1. New Building for Mathematics and Oceanography: the Central Court from Walnut Street

graduates. There are four main laboratories, two for physical work and two for chemical. Other rooms, such as a spectrographic room, balance room, dark room, workshop and store, are provided for special purposes. The laboratory accommodation and equipment offer facilities, on the chemical side, for work on the analysis of sea-water, particularly of trace elements by spectrophotometric methods, and on the composition of marine sediments. On the physical side, there are facilities for developing and testing apparatus for measuring, for example, the temperature and electrical conductivity of sea-water, and the velocity and turbulent fluctuations of currents.

At right angles to the single-storey wing, parallel to the main wing, there is a two-storey block containing, at one end, staff rooms and a combined library and chartroom for oceanography. The remainder of this block is occupied by the Sub-Department of Numerical Analysis, which possesses, in addition to rooms for its staff, others which house an electronic computer and its ancillary equipment, and a mathematical laboratory.

The building was designed to cater for the estimated number of science and engineering students, and the appropriate staff, likely to study mathematics in one or more of its aspects, or oceanography, within a total student community of 5,000. The erection of the building had scarcely been begun, however, when, under the stimulus of revised estimates of national requirements, the University agreed to plan its development on the basis of an ultimate student population of about 7,000. Large as it appears to be, therefore, the building will probably not be big enough to meet the demands likely to be made on it towards the end of the 'sixties.

The research carried out by the staff and graduate students of the Department of Pure Mathematics (Prof. A. G. Walker), the Department of Applied Mathematics (Prof. L. Rosenhead), with its Sub-Department of Mathematical Statistics (R. L. Plackett, reader in mathematical statistics), and its Sub-Department of Numerical Analysis (Dr. A. Young, reader in numerical analysis), and the Department of Oceanography (Prof. K. F. Bowden) covers a very wide range. The work that is going on in oceanography has been mentioned earlier; in pure mathematics research interests include classical analysis, group theory, algebraic and differential geometry and topology; and in applied mathematics work is being done on fluid motion, acoustics, quantum theory, nuclear physics, theoretical chemistry, astronomy, geophysics, numerical analysis and mathematical statistics.

The facilities of this well-co-ordinated group of teachers and research workers will be increased within the next few years when, with the aid of the grant recently promised by the University Grants Committee, an English Electric *KDF*9 Computer will be obtained to replace the existing *Deuce* of the Computer Laboratory, which was bought with funds provided by local industrialists and the University Council.

The University skyline at the top of Brownlow Hill is changing significantly—new buildings for physics, chemistry and veterinary science have been erected; still more buildings are going up for chemistry—and the foundations of engineering buildings as well as those of an extension of the Students' Union are now being laid. Within this complex of building activity the white-walled Mathematics and Oceanography Building stands out as something rather different and aloof; but the building will, we hope, serve as a university focus of mathematical thinking, teaching and research, and a centre from which mathematical service will flow to the University as a whole—as well as a place where important work on oceanography will be carried out.

L. Rosenhead

OXIDATION PROCESSES IN CHEMICAL MANUFACTURE

THE two-day symposium on "Oxidation Processes in Chemical Manufacture" organized by the London Section of the Society of Chemical Industry, was held in the William Beveridge Hall of the University of London during September 28–29, and attracted an audience of some 250 scientists from industry and the universities—with many overseas members.

The remarkable development in industrial oxidation processes over the past decade is perhaps best illustrated by the fact that while six comparatively new processes were discussed in some detail by the speakers, an equal number were mentioned only in passing. Among the latter were the direct oxidation of ethylene to ethylene oxide, the oxidation of propylene to acrolein, the subsequent oxidation of acrolein to glycerol, the direct oxidation of propylene in the presence of ammonia to acrylonitrile, and the production of hydrogen peroxide by the peroxidation of isopropyl alcohol. The processes chosen for discussion were the direct oxidation of ethylene to acetaldehyde, the vapour phase oxidation of acetaldehyde to peracetic acid, the uses of hydrogen peroxide in industrial oxidations with particular reference to epoxide formation, recent developments in the oxidation of aromatic hydrocarbons and the recovery of chlorine by oxidation of hydrogen chloride. Theoretical work on liquid-phase oxidation of organic compounds, vapour-phase oxidation of hydrocarbons and liquid phase oxidation catalysed by soluble metal salts was also discussed.

The direct oxidation of ethylene to acetaldehyde in the presence of a palladium chloride–copper chloride catalyst was described by Dr. Smidt (Consortium für Elektrochemische Industrie G.m.b.H.) as a single-stage reaction possibly applicable to other olefines. The mechanism of the reaction in the case of ethylene involves the formation of a complex between ethylene and palladium chloride. The resulting complex decomposes to give acetaldehyde, the palladium metal being precipitated. Since the decomposition of the complex is inhibited by acids, it is suggested that the first step in the reaction is the addition of a hydroxyl ion to the complex-bound olefine. In the second stage the π electron pair of the olefine passes over completely to the palladium with simultaneous migration of an hydride ion from carbon 1 to carbon 2. A double bond is then formed between carbon atom 1 and the oxygen of the hydroxyl group with expulsion of proton. On this hypothesis the oxygen required for the oxidation

of the olefine is supplied by the water, and only the oxygen atom from the water passes over to the olefine during the reaction, all four hydrogen atoms originally present in the ethylene remaining in the aldehyde. This is in contrast to the suggested mechanism discussed by Moiseev and co-workers. Unpublished work of Dr. Kreckeler of Farbwerke Hoechst has, however, shown that decomposition of the dinuclear ethylene–palladium chloride complex by heavy-water yields acetaldehyde which does not show any deuteration in mass spectrographic examination.

The commercial development of the reaction to a continuous process required the precipitated palladium metal to be re-oxidized after reaction 1:

$$C_2H_4 + PdCl_2 + H_2O = CH_3CHO + Pd + 2HCl \quad (1)$$

$$Pd + \tfrac{1}{2}O_2 + 2HCl = PdCl_2 + H_2O \quad (2)$$

The addition of cupric chloride enables the second step of the process to be carried out since cupric chloride readily oxidizes the finely divided palladium and the cuprous chloride so formed is quickly re-oxidized to the cupric state. The overall reaction may then be written:

$$C_2H_4 + 2Cu^{2+} + 4Cl^- + H_2O \xrightarrow{Pd\ Cl_2} CH_3CHO + 2H^+ + 2(CuCl_2)^-$$

the cuprous chloride being reoxidized by air.

In the commercial process, as outlined by Dr. Smidt, ethylene reacts with the catalyst solution, under moderate pressure and medium temperatures, in a flow reactor to 99 per cent conversion. After releasing the pressure of the catalyst solution the acetaldehyde formed distils off (the reaction heat being sufficient to accomplish this), and the reduced catalyst solution is pumped to the oxidation reactor when it is regenerated by air and returns to the ethylene reactor. Further details of this elegant process will be awaited with great interest.

The development of a commercial process for the manufacture of peracetic acid by the vapour phase oxidation of acetaldehyde was described by J. A. John and F. J. Weymouth, of Courtaulds, Ltd., and British Celanese. The major problem associated with this development was the explosion hazard associated with the concentration of peracetic acid. Experimental work established that concentrations of peracetic acid in acetic acid below 40 per cent peracetic acid were stable and that in the vapour phase excess acetaldehyde reduced the hazard to safe conditions. Three pilot plants, each of increasing capacity, had been successfully operated and the process was now considered suitable for commercial exploitation. The major industrial usage of peracetic acid developed in recent years is as an epoxidizing agent for the production of epoxy soya bean oil, epoxy tall oil esters, di*cyclo*pentadiene dioxide and the diepoxide from vinyl *cyclo*hexene.

The production of hydrogen peroxide by the barium process or by electrolytic methods has been virtually replaced in Britain by the method based on the cyclic oxidation and reduction of 2-ethyl-anthraquinol. The development of an alternative method of production by direct oxidation of isopropylalcohol in the United States will lead to increased availability of this material. T. D. Manly, of Laporte Chemicals, Ltd. gave an account of the wide variety of industrially important chemical processes using hydrogen peroxide. Supplied in tank-car quantities stabilized against chance impurities the modern product can be used at all strengths under many different conditions without suffering detectable loss by decomposition.

The recent developments in the production of a variety of chemicals using aromatic hydrocarbons from petroleum or coal tar were outlined by R. Landau and R. W. Simon. The processes discussed included the manufacture of phthalic anhydride from naphthalene or *ortho*-xylene, maleic anhydride from benzene—or from butene although a direct comparison of these two processes was not given—liquid-phase oxidation of mixed xylenes to yield isophthalic, phthalic, terephthalic acids and the recently announced oxidation of pseudocumene to give trimellitic anhydride. Brief mention was also made of the many processes now available for the production of phenol from benzene and of newer processes using *cyclo*hexane and *cyclo*hexanol obtained by hydrogenation of benzene and phenol respectively, for the production of adipic acid.

An account of recent developments in the oxidative recovery of chlorine from hydrochloric acid by W. F. Engel, S. Muller and M. J. Wade of the Shell Laboratory, Amsterdam, introduced an inorganic oxidation process—the Deacon process—into the discussion. The increasing amounts of by-product hydrochloric acid produced in conjunction with the manufacture of chlorinated organic compounds coupled with the probability of an increase in the price of chlorine as the demand for chlorine increases beyond the so-called caustic-chlorine balance—has stimulated development of processes for the recovery of chlorine from the acid. Three such processes have been announced recently, the De Nova electrolysis of hydrochloric acid operated by Monsanto, the oxidation by mixed nitric/sulphuric acid developed by the Institut Français de Petrole and the modified Deacon process described by Shell. Several companies have suggested the use of a fluidized-bed technique using modified cupric chloride catalysts. The Shell process employs a modified cupric chloride catalyst supported on silica in a fluidized bed at temperatures between 350° and 360° C. using air or oxygen. Conversions of 76–77 per cent per pass are claimed. On the figures presented the economics of the process are attractive.

Theoretical aspects of oxidation process were reviewed in three papers. Liquid-phase oxidation by molecular oxygen was reviewed by G. H. Twigg with particular reference to the oxidation of hydrocarbons and the reactions involved in the oxidation of cumene to phenol and acetone. The extension of the general reaction mechanism to oxidation of aldehydes, ketones, alcohols and olefines while generally accepted does not entirely satisfy, and further work in this field is desirable. Recent work on the oxidation of binary mixtures is likely to be of considerable theoretical interest. The gaseous oxidation of organic compounds was discussed by C. F. Cullis with particular reference to the effects of temperature, pressure, surface catalysis and mixture composition on the course of oxidation of a particular starting material. Investigations such as those reported on the vapour-phase oxidation of acetaldehyde to peracetic acid indicate that by careful choice of reaction conditions the reaction may be controlled to give, as final products, compounds which normally would occur only as intermediates in a more general oxidation. It is, however, doubtful if the knowledge of low-temperature vapour-phase oxidation processes is sufficiently advanced to enable prediction of reaction conditions in particular cases to be made on the basis of generalized theory.

XUM

The oxidation of organic compounds by metal ions and complexes was reviewed by H. R. Bacon with particular reference to complexes of silver and copper. Recent work using argentic piccolinates to catalyse the oxidation of primary and secondary amines has indicated that the effectiveness of the metal ion may be modified by the nature of the ligand. Comparison of reactions catalysed by cobalt, silver and copper complexes may lead to a better understanding of the reaction mechanism.

The conference concluded with a general discussion of the relative merits of fluidized-bed versus fixed-bed reactors for oxidation reactions. For those reactions, such as the modified Deacon process, in which thermal balance is readily achieved, the fluidized process is preferable. In reactions liable to rapid heat evolution, leading to possible explosion hazard, fixed-bed reactors may be preferred although sufficient data on the successful application of the fluidized technique to such reactions are not yet available. F. Morton

FAST REACTIONS IN PHYSICAL CHEMISTRY

THE first of the triennial Cambridge Physical Chemistry Summer Schools was held in August 1947 but the sequence was broken after 1956 by a gap of five years.

Each school has been organized by Prof. R. G. W. Norrish and his colleagues in the Department of Physical Chemistry in Cambridge and this year Dr. A. B. Callear and Dr. B. P. Levitt acted as organizers. The topics were selected to form a composite pattern of ideas related to current researches in the Department. Certain lecturers belonged to the Department; others were guest lecturers.

The intention has always been to correlate facts and notions within the pattern chosen, to stimulate discussion, to invite, even to provoke, in a friendly way controversy, rather than to instruct by presenting the facts, figures and theory as finished uninspiring blocks of knowledge which could well be posted in cyclostyled form. The pattern for the 1961 School was "The Study of Fast Reactions".

Dr. T. M. Sugden (Cambridge) dealt effectively with the recent developments in the collision and transition state theories, a matter of constant concern with so many chemists in industry and college alike. In his energetic, elusively 'off the cuff' manner, he analysed the processes of energy exchange with special reference to fast reactions, unimolecular decomposition of polyatomic molecules and the recombination of atoms. It may be well imagined that there was much animated discussion during the half-hour coffee-break.

Dr. Sugden described the photometric investigation of flames. He referred to experimental techniques for the measurements of H-atom, O-atom and OH— group concentrations in flames. Significances of the method included the determination of bond strengths in molecules such as M—Cl, M = Mn, Ni, etc. Trends into the future were not neglected. In another talk, Dr. Sugden dealt with chemical reactions in the upper atmosphere. Methods of investigation of temperature, density, pressure, and composition gradients and the reactions occurring in different strata are bound to attract quickly growing interest in coming years.

Reactions in shock waves were discussed by Dr. A. G. Gaydon (Imperial College of Science and Technology), who outlined experimental methods of observing the emission spectra and temperatures of shock-heated gases. Vibrational relaxation times of nitrogen and carbon monoxide, and the measurement of dissociation-rates of oxygen, hydrogen and carbon dioxide were other points of lively interest to the non-expert as well as to the expert listener. In another lecture, Dr. Gaydon discussed the advantages of low-pressure flames for the study of reaction zones, referring to the identification of reactive intermediates by emission spectra of flames, to the measurement of temperature by study of rotational structure of bands, the reversal of spectral lines and to reactions in hydrocarbon flames.

Chain reactions in gases, especially those leading to the ignition of gases, were discussed by Dr. P. G. Ashmore (Cambridge), who has taken part in a number of previous Schools. Theories of ignition limits and of induction periods were discussed with reference to non-branching and branching chain reactions.

Ultra-violet spectroscopy and fluorescence was dealt with by Dr. A. B. Callear (Cambridge), who opened with a helpful reminder on the use of term symbols and mode of coupling of angular momenta in atoms. Information on spin-orbit relaxation can be gained from examination of the quenching of the fluorescence of sodium and mercury. Rate of vibrational relaxation of electronically excited I_2, S_2 and NO was referred to.

Evidence for ions in flames was presented by Dr. D. R. Jenkins (Cambridge), who outlined experimental methods for the determination of distribution of ions by means of Langmuir probes, microwave and radio-wave attenuation and mass spectroscopy.

Relaxation in liquids was the title of a lecture by Prof. J. Lamb (Imperial College of Science and Technology), who outlined the fundamental theory of acoustic relaxation to what must have been many new attentive ears. The potentialities of the method were amply illustrated in a very convincing way.

Techniques for the measurements of fast reactions in solution were considered by Dr. R. H. Prince (Cambridge), who gave a lucid survey of mechanism and reactivity revealed by a study of fast reactions in solution. Substitution reactions in organometallic and co-ordination compounds; redox reactions, rapid isotope exchange were mentioned in particular.

Kinetics of fast reactions in solution was the title of the lecture by Dr. E. A. Moelwyn-Hughes (Cambridge), who, in his inimitable and stimulating way, brought freshness to the ever-difficult problems of rate data and transition-state characteristics. Interpretation of rate data as a function of temperature, certain limitations of the transition state formulation and the derivation of equations used in the interpretation of relaxation of ionic equilibria were critically commented on.

The lecture on fast electrode reactions by Dr. G. C. Barker (Atomic Energy Research Establishment, Harwell) was a very helpful contribution. He considered the theoretical basis of the measurement of rapid redox reactions which occur at the electrolyte/electrode interface and remarked in useful detail on

experimental methods involving single-step square-wave pulses and Faradaic rectification.

Not quite in the pattern but timely and very helpful was the talk on high-speed gas chromatography by Dr. J. H. Purnell (Cambridge). Factors determining the correlation between the speed of a gas chromatographic analysis and the efficiency of the process were examined in a cursive and conciliatory way, appropriate in dealing with a technique which is still much of an art. If necessary, remarkably short analysis times, less than 1 sec., could be achieved, and are attractive in the study of gas reactions.

Work on isothermal reactions of atoms and radicals by flash photolysis was discussed by Prof. R. G. W. Norrish, who illustrated the applicability of the techniques of flash photolysis in the detailed scrutiny of very fast reactions in gases. In the last lecture of the School, he went into the matter of the chemistry of combustion as revealed by kinetic spectroscopy, showing the applicability of flash photolysis in the investigation of adiabatic processes. Anti-knock action of tetraethyl lead was related to the appearance and disappearance of lead oxide and lead during explosion.

Time for discussion was allowed at the end of each lecture and indeed there were some astute points put. There was animated and valuable discussion during the long coffee-break in the morning and during the afternoons, which were in the main devoted to visits to the laboratories and to a lecture at 5–6 p.m. There were social meetings when easy exchange of ideas was stimulated.

An outstanding feature was the well-organized social life of the School, and in this Mrs. Norrish and other ladies had doubtless been influential. There were some 120 members from industry and colleges and quite a number were from overseas.

W. GERRARD

METALLURGY OF BERYLLIUM

BERYLLIUM has only emerged as a metal of industrial significance during the past few years. Even now, its use is not securely established and is faced by a number of real difficulties. The following assessment of its present position is based on seventy papers presented at an international conference arranged by the Nuclear Energy Committee of the Institute of Metals and held in London during October 16–18.

The potential applications of beryllium at present lie in two main fields—aircraft, missiles and spacecraft on the one hand, and atomic reactors on the other. In the first, the advantageous properties of beryllium are its high-strength-to-weight ratio, high elastic modulus, specific heat and thermal conductivity and moderately low coefficient of expansion. With a density of 1·85 and a tensile strength of 50–70,000 lb./sq. in., beryllium has a superior strength/weight ratio to all other metals up to about 350° C., although some of the recently developed high-strength stainless steels are not far behind. However, this is not beryllium's greatest attraction. Elastic stiffness and resistance to buckling under compression rather than tensile properties are the limiting factors in many design problems in this aeronautical and spacecraft field, and it is in this that beryllium's combination of low density and high modulus of elasticity ($E = 43 \times 10^6$ lb./sq. in.) is outstanding. Thus, for example, a box beam designed in beryllium to resist a buckling load at up to 450° C. is only about half as heavy as one in titanium alloy and about one-third as heavy as one in a high-strength stainless steel. In addition, the high specific heat and thermal conductivity and the low coefficient of expansion are important in minimizing the effects of aerodynamic heating. With these properties, "beryllium holds one of the keys to a successful flight to the moon" (I. Perlmutter, Wright-Patterson Air Force Base).

For atomic reactors, on the other hand, the attraction of beryllium lies in its low neutron adsorption, and at present there is wide investigation of its possible use as the fuel element can in high-temperature, gas-cooled reactors. In addition, the Australian Atomic Energy Commission is examining the use of beryllium, instead of graphite, as a moderator in low-power reactors on the grounds that this would permit smaller reactor cores and possibly reduce over-all cost. It is also considering fuel elements in which the fuel, for example $(U,Th)Be_{13}$, is dispersed in beryllium.

Unfortunately, these attractive properties are accompanied by two immediate disadvantages. Beryllium is very costly; billets are about £20 per lb. The United Kingdom is not a producer; supplies come from Pechiney and from the United States. Secondly, there is a severe health hazard. Inhalation of the metal or its compounds produces acute chemical pneumonitis, or, at lower doses, a chronic lung disease, beryllosis. To keep the beryllium content of the air at safe levels, special workshops are required with air extraction and filtration, and total enclosure of some machines. Monitoring of the beryllium-levels and health checks are necessary. However, the applications suggested for beryllium are those that can withstand a fairly high cost and, once a beryllium-handling facility has been established, the health-hazard is not too troublesome. There remains a third disadvantage, that of brittleness, and at present this is the major problem in the metallurgy of beryllium. Practically every paper read at the conference in London was to some extent concerned with brittleness.

Beryllium is quite ductile at normal hot-working temperatures of 1,000°–1,050° C., so hot-fabrication processes do not present any great difficulty. However, below about 200° C. there is a fairly sharp transition to a low ductility. This not only means that fabrication by cold-work is difficult or impossible, but also, and more important, that in service high stresses, perhaps purely local, that might be relieved by plastic distortion, lead instead to fracture.

This transition to brittleness is accompanied by a transition in fracture mode from so-called 'ductile fracture' to a process of separation along definite crystallographic planes, the cleavage planes. Thus, the brittleness of beryllium appears to correspond to the classical brittle fracture of mild steel that has caused so much concern and investigation since the 'Liberty' ship failures during the War.

Investigations of mild steel have brought out the importance of two stages in the development of cleavage. First, the initiation of a crack by some

XUM

process such as the coalescence of the dislocations in a slip plane through compression against a grain boundary, or by the coalescence of dislocations through interaction with others on an intersecting slip plane or by twinning. Secondly, the break-out of this dislocation crack across a cleavage plane in the manner of a Griffith crack propagated by the externally applied stress. It is considered that the transition to cleavage occurs when the criterion for propagation is fulfilled.

This criterion involves three factors. First, the size of the dislocation crack depends on the number of dislocations forming the crack and therefore on the grain size. The smaller this size, the smaller the dislocation crack and the more difficult is cleavage. Secondly, the effective surface energy associated with crack growth represents partly the true surface energy and partly the plastic work arising from the operation of dislocation sources in the region of stress-concentration surrounding the growing crack. The easier dislocation movement, the greater the amount of plastic work, the shorter the initial dislocation crack and the more difficult its propagation. Thus, a high effective surface energy arising from easy dislocation movement favours ductility. Thirdly, the applied stress available for crack propagation is determined by the flow-stress at the instant of fracture. The reason why low temperature favours cleavage is that the flow-stress is then higher and the effective surface energy lower because dislocation movement is more difficult. The papers at the conference showed that the conclusions from the work on steel have now been applied at least qualitatively to beryllium.

The attempt to overcome brittleness by a fine-grain size at present divides beryllium work into two categories, powder and ingot. Present industrial producers of beryllium components start from powder, and the oxide derived from the coat on the particles inhibits the growth of grains during sintering and annealing. In this way, the grain size can be kept to about 50μ. Others, notably the group headed by G. C. Ellis, A. J. Martin and A. Moore at Aldermaston, argue that powder methods yield an intrinsically poorer material with reduced high-temperature ductility and resistance to corrosion and they have put their effort into an attempt to obtain fine grain size in wrought material derived from an ingot. The difficulty lies in the easy growth of grains of this material on re-crystallization. So far, the finest grains, 40μ, have been obtained by 'flash annealing'; that is, exposure for a few seconds to a temperature of 900°–1,000° C., well above the normal re-crystallization temperature, so that many nuclei are active. Such treatment is only possible with thin sections. However, even by normal re-crystallization it has now proved possible to keep the grain size at 60–80μ in material that has been worked as far as sheet. A full assessment of the mechanical properties of the products derived from ingot has not yet been obtained.

Another possible approach to reduced brittleness is through purification. This may be beneficial because of a reduction in the flow-stress available for crack propagation and an increase in the effective surface energy through easier dislocation movement. The beryllium obtainable commercially is not particularly pure; it contains a few hundred p.p.m. of various metals, mainly iron, aluminium, silicon and of non-metals, oxygen, carbon and nitrogen. A number of laboratory-scale attempts at purification by distillation and zone-refining were reported at the conference. A. R. Kaufmann *et al.*, for example, reduced the contents of the metallic impurities to the 1–5 p.p.m. range. There appeared to be a reduction in flow-stress and increase in ductility. However, the purification is difficult and consolidation of the product to a uniform, fine-grained polycrystalline state has not yet been achieved. Thus, at the moment there does not seem to be an immediate easy path to ductility through purification. Perhaps in any event it may be unsafe to expect too much from this approach in view of the thin-film electron microscope work of H. G. F. Wilsdorf and F. Wilhelm that showed strict confinement of the dislocations to crystallographic directions, suggesting that the intrinsic lattice resistance to dislocation motion (the Peierls–Nabarro stress) is already high, independent of impurity hardening.

Although the brittleness of beryllium seems to be essentially similar in principle to that of mild steel, it emphasizes a point that is not important with steel. Beryllium is close-packed hexagonal and suffers from a dearth of slip systems. At room temperature, slip on the single basal plane is by far the easiest; (10$\bar{1}$0) slip is 4–5 times as difficult. Neither of these contributes any component in the *c* direction. Thus, any one grain in a polycrystal experiences difficulty in deforming crystallographically in conformity with the matrix. Consequently, as brought out by J. Sawkill and by B. Allen and A. Moore, there is bending and kinking of the slip planes and this supplies, at least in some cases, the fracture nucleation mechanism. This is the one suggested a number of years ago by Orowan and by Stroh in which the discontinuity in displacement when slip crosses a bend plane produces cracking down the slip plane. A second effect of the dearth of slip planes is that wrought products such as sheet or extruded bar, which have received highly directional working, are strongly textured with the basal planes parallel to the direction of working. This has a marked effect on ductility. For example, cross-rolled sheet can show 30–40 per cent elongation in the plane of the sheet even at room temperature, but there is practically no ductility in the third dimension and such sheet readily fractures in forming processes. Thus, the control of texture is an important aspect of the brittleness problem. Some success in achieving a more random orientation in sheet has been obtained by the press-forging of beryllium encased in a steel container and afterwards rolling the flat produced (R. L. Craik and D. A. Barrow).

Another interesting approach to the problem of brittleness has been attempted at the Armour Research Foundation, Chicago. This is to use 'liquid-phase sintering' as in the common tungsten carbide–cobalt tools. The beryllium is dispersed in a continuous plastic matrix of silver–aluminium–germanium that is liquid at the sintering temperature and spreads around the beryllium particles. The product is quite ductile at room temperature, but Young's modulus is reduced to 22×10^6 lb./sq. in.

In addition to low-temperature cleavage, beryllium also shows a dip in ductility around 600° C. due to the occurrence of intercrystalline fracture. This is not so important as cleavage, and in any event it has now been shown at Aldermaston and other centres that annealing about 800° C. followed by slow cooling removes the brittleness produced at 600° C. The heat-treatment appears to over-age impurity precipitates and restores a proper balance between the ease of deformation within a grain and at a grain boundary.

Apart from this brittleness problem, two other problems appear when beryllium is used in a reactor. In the carbon dioxide–water coolant mixtures, breakaway, that is, accelerating, oxidation can occur. The papers at the conference did not add a great deal on this topic, although the Australian Atomic Energy Commission reported an inhibition of breakaway by oxidation of the beryllium powder at 800° C. in oxygen prior to sintering. Irradiation damage also occurs in a reactor. The normal displacement of atoms by neutrons that is responsible for the damage in steel and copper has little effect in beryllium, and damage only appears at doses above about 10^{19} neutrons cm.$^{-2}$ when it is due to the helium generated by neutron reaction with beryllium. R. S. Barnes and others have shown that when present as small bubbles the helium leads to hardening and loss of ductility. Larger bubbles accumulate at the grain boundaries and these promote fracture in creep. They can also give grain boundary permeability, which is, of course, highly undesirable in a fuel can.

It is perhaps clear that there is no lack of beryllium problems. However, considering the advances that have been achieved in the quite short period of really active interest, the future is not without hope.

N. J. Petch

CHANGES OF CLIMATE

As was entirely appropriate for a symposium sponsored by the Unesco major project on scientific research in arid lands, an effort was made on the occasion of the recent meeting in Rome (October 2–7), arranged by Unesco and the World Meteorological Organization, to restrict the interest to climatic variations which have occurred since the latest glaciation, with particular attention to the period of the meteorological record. The reason behind this decision was no doubt the wish to talk about something which might conceivably have relevance to the nature and trends of the arid lands of to-day—relevance on the scale of economic planning, say, for a hundred years. At the same time this emphasis on what, after all, is micrometric on the geological scale served the purpose of almost eliminating the endless speculations on the causes of ancient climatic change, fascinating though they undoubtedly are. Most geological, geophysical and astronomical happenings which are potential climatic controls, such as mountain building and denudation, drifting of the continents, changes in solar constant and a score of other possible processes, are eliminated when the time-scale is a mere 100 or 1,000 years

It should not be supposed, of course, that examination of such recent changes will throw much light on events on the longer time-scale. The odds are that variability on each different scale of time presents an essentially different problem with an essentially different theoretical explanation. But it is good to keep a symposium within bounds, and the short-period problem is the most tangible, the most suitable for the attention of the theoretical meteorologist, and the most important economically.

More than forty papers were presented, divided into four sessions each of one day and treating respectively the period of the meteorological record (chairman, R. G. Veryard), the late geological and early historical period (chairman, K. W. Butzer), theories of recent climatic change (chairman, R. C. Sutcliffe) and significance of changes of climate (chairman, R. O. Whyte). A summing-up by C. C. Wallén on the last day was particularly well received.

The first session was much helped by the chairman's fine survey of the literature, and the papers presented did not seem to take us very much further with the facts, although substantiating the main features now becoming generally familiar, in particular the warming of extratropical latitudes over the century ending about 1940. J. M. Mitchell's world-wide view of the evidence will be found particularly useful, most of the other contributions being local studies. An effort was made to give attention to the statistical treatment of time series on the basis of a paper by R. Sneyers, it being well known that a good proportion of the published literature on climatic change suffers from uncritical and undefined statistical standards. The general technique of power spectrum analysis received mention but was not pursued. It was, however, evident that those in the field were very conscious of the problems.

The second session fell mostly to the non-meteorological disciplines, geology, geomorphology, archæology and dendrochronology being represented. Apart from the chairman's valuable introduction, the most persuasive paper was by R. W. Fairbridge on mean sea-level during the past 20,000 years. It is remarkable and a little puzzling that such clear evidence should be obtainable from data which one would have thought to be confusing.

Under the heading of "Theories of Changes of Climate", the papers were entirely by meteorologists, some of whom were new to the field. This was a good feature, as a weakness of the subject is its failure to attract expert meteorologists aware of the complex behaviour of the atmosphere. The chairman directed attention to the possibilities of variability through feed-back contained within the Earth–ocean system without necessarily recruiting the aid of extraneous factors, radiation, dust, carbon dioxide and the like, and other papers developed the theme: both J. Bjerknes and J. Namias were on this side. On the other hand, the most substantial paper was perhaps that by E. B. Kraus, giving the results of a heavy calculation nobly carried out on the effects of a radiation change on the general circulation. The paper was more an illustration of the mathematical approach than a practical solution, but was important as being one of very few attempts that have yet been published.

In the final session, L. P. Smith's paper on "The Significance of Climate Variations in Britain" was very much to the point, and anyone who previously had doubted whether the subject had any economic significance must have given way at this point.

The broad impression created by the meeting is that, for the period of the instrumental record, the subject, which has got to a certain descriptive stage by pure empiricism, is in danger of languishing for want of ideas. Perhaps the symposium marks a turning point, and the attention which climatologists are giving to statistical techniques and which theoretical meteorologists are giving to the general circulation are pointers to the future. For the longer record

the interest must continue to rest on techniques of exploration and interpretation of evidence, and here there is no slackening of interest, for most of the Earth's surface is almost unexplored.

The facilities provided by the Food and Agriculture Organization and the Italian authorities were admirable. After several experiences of large meetings, one looks forward to those where headphones and simultaneous translations are provided. To be able always to hear is a wonderful asset, even if the language presents no difficulty. It is understood that the complete papers are to be published by Unesco and this will be worth while. A good proportion of the material had been heard or read before, but there would be no sense in insisting on complete novelty for an interdisciplinary meeting where the object is to exchange ideas, not to be mutually incomprehensible.

R. C. Sutcliffe

CHEMICAL WARFARE ON PLANT PESTS AND DISEASES

THE first British Insecticide and Fungicide Conference organized by the Association of British Manufacturers of Agricultural Chemicals was held at Brighton during November 7–9 under the presidency of Dr. H. G. Sanders, chief scientific adviser (agriculture) to the Ministry of Agriculture, Fisheries and Food. It was truly an international gathering, for of the 470 delegates attending from official research stations, Government departments and manufacturing firms, nearly one-quarter came from fourteen countries overseas.

The conference consisted of ten successive sessions devoted to important present-day problems in plant pathology and their possible solution by chemical means. During the first two days each session began with one or more introductory papers by authorities on the subject under discussion, and these were followed by brief reports of relevant research in progress at various centres. The morning of the third day was given over to research reports on new insecticides, fungicides and acaricides, and on novel formulations as well as new methods of application. In the final session attention was appropriately directed to the problems involved in translating research into practice and in introducing insecticides and fungicides into agricultural practice both in Britain and overseas. In all, more than fifty papers or reports were presented from the research departments of official agricultural institutes and industrial firms, including a number from Holland, Germany and Italy.

On introducing the president, Mr. H. C. Mellor, the chairman of the Manufacturers' Association and of the conference, said that the industry is at present under fire, though much of the criticism levelled at it is uninformed. The members of his Association are as anxious as anyone to find safer chemicals, and are most careful to honour the agreements made on their behalf with the Ministry of Agriculture, as well as to satisfy the requirements of the Notification Scheme under which all new chemicals are notified to the Ministry for clearance before marketing.

In his opening address Dr. Sanders referred to the increasingly important part played by chemicals in farming. Unfortunately, some of them are toxic to mammals and wild-life, and this has led to alarm in the public mind. He believed that much of this concern is due to ignorance of the many hurdles new chemicals have to surmount before they are cleared for marketing by Government departments of health and agriculture. The present conference was mainly a scientific one, but the delegates included many whose functions were to translate science into practice, and it should therefore provide a good opportunity for getting things, including toxic hazards, into proper perspective. Dr. Sanders also commented on the close co-operation now existing between industry, research workers and Government officials, and expressed appreciation to the manufacturers for disclosing to him highly confidential information which has fully satisfied him that during the past five years there has been a very substantial reduction in the acreage sprayed with the more toxic chemicals, even though the total acreage sprayed has doubled during that period.

The first session was concerned with soil pests and diseases of agricultural crops, and included a review by Dr. Hubert Martin (until recently director of the Pesticide Research Institute, London, Ontario) of a symposium called privately, in February 1961, to discuss the effects of chlorinated hydrocarbons and other chemicals on the ecology of the soil fauna and flora. Dealing with the eelworm problem, Mr. F. G. W. Jones (Rothamsted Experimental Station) said that a first-class soil nematicide is still awaited. Only three—bromomethane, dibromoethane and dichloropropene—have stood the test of time, though some dithiocarbamates show promise. He doubted whether soil treatments were the right approach. Apart from difficulties of mixing and dispersion, at least 1,000 tons of soil per acre has to be treated. Eradication of eelworms is impossible and unnecessary, and he looked forward to a systemic nematicide moving from foliage to roots, suppressing the cyst-hatching factor, and perhaps passing out into the soil to exert a repellant effect. Dr. Mary Noble (Department of Agriculture for Scotland) considered that seed is not the best launching vehicle for a chemical intended to control soil-borne fungus diseases, while Mr. M. J. Way (Imperial College of Science and Technology) did much to restore a balance in the campaign against insecticidal seed dressings by insisting that the minute quantities used are effective and well placed and do not cause off-flavours in crops, injure beneficial insects or encourage resistance in the pests attacked. The problem of deaths of birds after eating dressed seed should be met by more circumspect use of seed dressings and not by restrictive legislation, a view that received much support.

The use of systemic insecticides for virus control in potatoes and sugar beet was considered at a second session. Hitherto, potato virus diseases have been controlled through crop certification schemes, a method that has become traditional. Dr. L. Broadbent (Glasshouse Crops Research Institute) and others have shown that similar ends may be achieved by using systemic insecticides, though few farmers have yet attempted to take advantage of this. With sugar beet there has been no tradition of certification, and farmers responded quickly to a spray-warning scheme begun in 1957 to deal with virus yellows. Dr. R. Hull

(Rothamsted Experimental Station) disclosed that 95 per cent of the crop was sprayed in 1959 and suggested that spraying had converted 1961 from a 'severe' to a 'marginal' yellows year. Other reports about this disease dealt with the value of menazon and dimethoate as sprays, and with the application of granular formulations of phorate and thiodemeton to the soil or foliage.

In a session on forecasting pest and disease outbreaks great interest was shown in the pioneering work of Mr. G. H. Brenchley (Ministry of Agriculture, Fisheries and Food) on the use of aerial infra-red photography to detect the primary foci of potato blight and to study patterns of its early natural spread. Dr. E. Evans (Fisons Pest Control, Ltd.) reported on difficulties in evaluating materials for blight control and suggested that the performance of copper products and dithiocarbamates differs according to the nature of the epidemic.

Three sittings were devoted respectively to apple mildew, fruit tree pests and the control of *Botrytis* diseases. It was obvious some years ago that apple mildew would again become the problem it now is, and older delegates listening to the seven speakers no doubt recalled similar discussions held thirty years and more ago. In the meantime, mildew has been adequately controlled under a sulphur programme for apples, but it became resurgent as captan and organo-mercury sprays replaced sulphur for scab control and as the renewal system of pruning evolved. Much of the discussion revolved around the difficulty of dealing with continuous conidial inoculum and preventing bud infection, which can evidently be fully accomplished only by weekly or even more frequent application of dinocap or other appropriate fungicides. On fruit tree pests, Dr. G. H. Dicker (East Malling Research Station) and others emphasized the importance of a knowledge of the biology of red spider mite, codling moth and tortrix in the effective timing of fruit tree sprays during the flowering period. No specific methods emerged for dealing with species of *Botrytis*, notably *B. cinerea*, which causes so much destruction to many crops, and the prospect was not made brighter by the ease with which Dr. R. K. S. Wood (Imperial College of Science and Technology) has been able to produce *in vitro* strains of *Botrytis* highly resistant to the fairly effective chlorinated nitrobenzenes. To protect strawberry and raspberry fruits, Dr. W. R. Jarvis (Scottish Horticultural Research Institute) felt that the only real solution would be in a systemic fungicide applied before the flowering stage.

In a session on soil sterilants, Dr. W. Madel (CELA, G.m.b.H.) described experience in Germany with metham-sodium against harmful soil organisms, and Dr. L. P. Flipse (Ligtermoet and Zoon N.V.) showed how valuable this fumigant and herbicide is to Dutch horticulture, particularly in controlling soil-borne fungus diseases of bulbs, carnations and tomatoes. But though practical results continue to accumulate, little is yet known about the mode of action of soil sterilants.

Comment on much of the work discussed at the conference must perforce await publication in the *Proceedings*. Other items included progress reports on a number of new aphicides and acaricides, on the potentialities of organic tin compounds and polybutene formulations, and on special formulations for low-volume spraying. In all the developments outlined it was not difficult to perceive a general desire to find effective chemicals of low mammalian toxicity (if possible, systemic in action) having prolonged effects, and preferably applied in a form that reduces hazards to man, animal and wild-life to the absolute minimum. It was also encouraging to learn from the Laboratory of the Government Chemist of the progress being made in solving the problems which face the analyst examining foodstuffs for pesticide residues.

Speaking as an adviser concerned with the translation of research into practice in Great Britain, Dr. M. Cohen (Ministry of Agriculture, Fisheries and Food) emphasized the difficulty that faces everyone confronted with the vast number of new materials produced during the past fifteen years, and referred to the apparently insoluble problem posed by the confusing combination of endless chemical, common and proprietary names for insecticides and fungicides on the market. Finally, and in an absorbing talk that was delightfully refreshing at the end of three days of "sharing the benefits of new knowledge", Mr. G. Ordish (Department of Technical Co-operation, Tropical Products Institute) dealt with the introduction of insecticides and fungicides into agricultural practice overseas. Little had been said at the conference about economics, though in his view this was perhaps the most important factor of all. In his closing remarks the president mentioned some of the highlights of the discussions and expressed the hope that the conference would be but the first of a biennial series.

W. C. Moore

THE SOVIET HYDROMETEOROLOGICAL SERVICE

A DECREE, signed by Lenin in June 1921, was the virtual foundation of the modern Soviet Hydrometeorological Service. In a recent issue of *Meteorologija i Gidrologija* (No. 6, 1961) the present chief director, A. A. Zolotuhin, traces the history of the Service over the ensuing forty years.

There had been a small meteorological service in Imperial Russia, but owing to foreign and civil war it was in a state of complete decline by 1921. Progress, however, was slow and inadequate during the 'twenties, and by 1929 there were twelve meteorological services in the U.S.S.R. which arose from departments forming their own services, because the main service could not meet their demands. The U.S.S.R. has for long undoubtedly regarded a soundly administered and technically efficient hydrometeorological service as essential to its national economy and to its defence. So in the early 'thirties a unified service was recreated and thereafter progress was rapid. For example, it is claimed that the world's first radiosonde was released in January 1930, and that a network of sounding stations was immediately commenced. By 1941, at the outbreak of War, forty radiosonde stations were in being. Advances in other directions were also substantial.

This was, of course, a period of great expansion for many national meteorological services owing to the development of the science itself and to the increasing demands of military and civil aviation. The U.S.S.R. was clearly no exception.

Since the War, the Hydrometeorological Service has been greatly expanded further. Separate specialist branches have been set up for particular branches of the economy. Emphasis is placed on activities of special importance to the economy of the Union, for example, civil aviation, the hydrology of rivers and watersheds, agricultural meteorology. Nevertheless, the confession is made that the rate of improvement in the accuracy of the forecasts has not really been adequate to satisfy its needs.

Dr. Zolotuhin, however, makes the point that it is the level of scientific research which determines the quality of the service, and there are now a considerable number of meteorological, hydrological and oceanographical research institutions in the U.S.S.R., and the Hydrometeorological Service also has a number of specialist educational establishments for training its staff and potential staff.

From the complete decline of 1921, the past forty years have clearly been years of major expansion; an expansion which indicates the importance attached by the U.S.S.R. to the sciences of meteorology and hydrology. Indeed, with its 29 local and republic directorates and its 20,000 observing points, embracing one-sixth of the Earth's land surface, the Soviet Hydrometeorological Service must now surely be about the largest in the world.

R. F. Zobel

POPULATION TRENDS IN THE U.S.S.R. AND THE UNITED STATES

In terms of population, the Soviet Union and the United States are the third and fourth largest nations in the world. Their combined population of approximately 402 million is close to the population of India, but only slightly more than half that of China. The two nations are alike in many demographic respects. A recent survey entitled "Population-Trends in the U.S.S.R.", issued by the Population Reference Bureau, Inc., compares the various population trends in the U.S.S.R. with those in the United States*.

In 1959, the Soviet census counted 209 million people. The birth-rate was 25, the death-rate 7·6 per thousand. In 1959, the United States population was 176 million, and the birth-rate 24·1 and the death-rate 9·4 per thousand. The age-specific death-rates in the U.S.S.R. were higher than those of the United States below age forty, and lower above that age. In both countries, the populations are made up of many ethnic groups; illiteracy is virtually non-existent, and great numbers of people are receiving more education. Only 1·5 per cent of Russians aged 9–49 were reported by the census to be illiterate, but there are urban–rural differences at every school-level. The educational pattern is very different in the two countries.

Migratory trends are similar. With the growth of industrialization, the people of both nations have been on the move, mainly from the agricultural areas to the urban areas. The force which motivates this migration is mainly the desire for advancement.

In 1926, 82 per cent of the population in the U.S.S.R. lived in rural areas and 18 per cent lived in cities. By 1959 the proportion had changed to 52 per cent rural and 48 per cent urban. Russians have been moving east in response to a Government-planned effort to colonize the 'new lands' where industrial complexes have been established.

In contrast, Americans have been going west and mainly into urban areas in a wholly unplanned, casual manner because they desire to live and work there. In 1900, 60 per cent of the population of the United States was rural and 40 per cent was urban. By 1960, the proportion had changed, 37 per cent being rural and 63 per cent urban.

There is a vast difference in the kinds of occupation in the two countries. In 1959, almost 39 per cent of the total labour force in the U.S.S.R. was engaged in agriculture. In 1960 less than 9 per cent of the labour force in the United States was so employed.

* Population Reference Bureau, Inc. *Population Bulletin*, Vol. 17, No. 6 (October 1961): Population Trends in the U.S.S.R. Pp. v+76. (Washington, D.C.: Government Printing Office, 1961.) 45 cents.

NEW SYNTHETIC DETERGENTS

Although the biologically 'soft' detergent which has been developed by the manufacturers, and has been widely distributed in the south of England, has eased the difficulties which have been caused by the discharge of detergent residues to surface waters, the new material does not afford a complete solution to the problems arising from the widespread use of synthetic detergents, and further improvement of this material or the development of new types of surface-active materials is necessary. This is the gist of the Fourth Progress Report of the Standing Technical Committee on Synthetic Detergents*. The new 'soft' detergent, originally supplied to the Luton area, has now been distributed to a large part of southern England, and increased removal of detergents at sewage treatment plants has been observed in many districts. No similar change has taken place in the north, where the type of detergent on sale has not been altered.

One of the difficulties encountered in experimental work on the breakdown of synthetic detergents is the lack of analytical techniques which can be used to study the breakdown products. The analytical methods used are specific for molecules of a given range of size and structure, and it is possible that during sewage treatment some molecules may be only partly degraded or otherwise changed in structure, and may then be undetected in the effluent. It is therefore emphasized in the report that figures given for percentage removal do not necessarily imply that the material has been fully degraded. An extensive programme to be undertaken by various members of the Committee will include investigations of the biological breakdown of single substances, in order to throw light on this part of the problem.

Nora H. Johnson

* Ministry of Housing and Local Government. *Fourth Progress Report of the Standing Technical Committee on Synthetic Detergents.* Pp. 19. (London: H.M.S.O., 1961.) 1*s.* 6*d.* net.

PRODUCTIVITY IN LETTERPRESS PRINTING

THE mounting cost of printing would alone give wider interest to the report on *Productivity in Letterpress Printing*, recently published by the Department of Scientific and Industrial Research*.

The report describes the results of an investigation made during 1956–57 by the Printing, Packaging and Allied Trades Research Association under a contract financed from Conditional Aid Funds and in which eighteen firms collaborated. The organization, methods and production of seventeen firms were examined in detail, and a detailed breakdown of machine time indicated that, on average, machines are only running for about 40 per cent of their manned time. Speed is accordingly not of prime importance: it is usually easier to increase production by reducing delays, make-ready times and other stoppages, and the average speed of new machines with a high potential running speed is little better than that of pre-war machines. What is needed in printing machines at present is the provision of devices to facilitate make-ready, control and adjustment.

The factors responsible for the low running time are discussed in the report, and while in some firms particular operations are inefficient, the greatest improvement can only result from a thorough examination of the printing process as a whole. Waiting time averaged 15 per cent and make-ready time 45 per cent of available time, and although the latter is a necessary and chargeable operation in printing, it can be substantially reduced by improvements in methods and organization. These usually could be made with little capital outlay but require an industrial engineering approach using work-flow principles, greater specialization of work by employees, quantitative measurement and mechanical aids for handling, and the like. At present, for example, so little method study is used that employees can earn bonuses on inefficient and even unnecessary operations, and conditions, as regards lay-out, in most machine rooms are described as chaotic.

The report is also critical of organization, production planning and storage in the industry, where also major changes in procedure are recommended. It is recognized that not all factors influencing efficiency are within the immediate control of printers, but there is scope for more standardization and greater co-operation between printers and other sectors of the industry. Better production planning and greater precision at each stage of the process could effect a real increase in production at lower costs, and a separate chapter in the report outlines the problems requiring further investigation. These chiefly call for operations research and kindred methods; very few purely technological problems came to light.

* Department of Scientific and Industrial Research. *Productivity in Letterpress Printing*. Pp. viii + 65. (London: H.M.S.O., 1961.) 5*s*. net.

A SPECTROSCOPIC SURVEY OF ENGLISH AND CONTINENTAL MEDIEVAL GLAZED POTTERY

By L. C. THOMAS and J. W. G. MUSTY

Chemical Defence Experimental Establishment, Porton Down, Nr. Salisbury, England

ONE of us (J. W. G. M.) recently claimed[1] that a medieval glazed jug from Bergen, found during the excavation of the Hanseatic Wharf there[2], had been made in the late thirteenth century kilns at Laverstock, near Salisbury, Wiltshire, England[3]. This and other attributions, based on a visual comparison of pottery sherds, have been checked by the spectroscopic examination of sherds from these sites and from a number of other English and Continental sites. While a range of sites has been covered, the number of samples from any site, other than Laverstock, has been limited. The variation in glaze composition found between samples from different kilns at Laverstock clearly indicates that more samples from each site must be examined for the full value of this technique to be realized. However, this preliminary survey has provided sufficient evidence to enable some conclusions to be drawn.

The pottery samples examined, other than those from Laverstock, were supplied by Mr. Gerald Dunning and included sherds from English kiln sites at Brill (Bucks.), Cheam (Surrey), Ham Green (Glos.) and Tyler Hill (Kent). Pottery found at Winchester and at Clarendon Palace, near Salisbury, was also examined as the kiln sites for these samples are unknown and Laverstock was thought to be one of the possible sources of supply. Examples of pottery attributed to foreign kilns, but found in Britain, included French polychrome ware from Glastonbury and Dover, French pottery found at Bristol, and the Belgian Andenne ware found at London and Canterbury. Samples of Andenne ware from Antwerp and from an Andenne kiln[4] were also examined. Samples found at Oslo and Bergen but attributed to British kilns were also included in this preliminary survey.

Small areas of the glaze were removed from the sherds by careful scraping with a tungsten carbide tipped masonry drill. Samples of the fabric were obtained from the unglazed side of the sherds as a control on the inevitable contamination of the glaze by the fabric. The samples thus obtained were ground in an agate mortar, packed into special 'cathode layer' graphite electrodes, and their spectra obtained and the line intensities evaluated using techniques previously described[5]. The results obtained will be reported in detail elsewhere. No attempt has been made to place these qualitative results on other than a semi-quantitative basis. Variations in the relative concentrations of elements have been assessed by variations in the relative intensities of lines of these elements in the same spectrum. The following elements had lines suitable

for evaluation in the region of the spectrum (2640–4300 Å.) examined: silver, aluminium, calcium, cobalt, chromium, copper, iron, potassium, magnesium, manganese, sodium, nickel, lead, silicon, tin, titanium, vanadium, zinc and zirconium.

Of these elements the only ones which the spectroscopic evidence proved to be significantly more concentrated in the glaze than in the fabric were silver, lead and tin. The evidence also suggested that the concentrations of copper and vanadium were higher in the glaze, but for these elements the evidence was not unequivocal. The use of lead in pottery glazes is, of course, well known, and the presence of tin in medieval glazes has previously been recognized by Jope[6,7]. As no reason for the deliberate addition of silver is apparent, it appears probable that this element occurs as an impurity in one of the other additives. While lead is the obvious choice for the silver carrier, the relative concentrations of lead and silver fluctuate too widely in closely related glazes for this suggestion to be readily accepted. The variation of silver content more closely follows that of the variation of tin, and this may indicate that some alloy or mineral containing both these elements has been added to the glazing material in addition to the galena normally accepted as the source of the lead. Both solder and pewter have been suggested by Jope[7] as possible sources of tin in glazes from medieval pottery.

The presence, and amount, of tin in the glaze has been used as a check on some of the accepted attributions of origin of the pottery sherds. All the samples examined of glaze from Laverstock kilns have a high tin content. However, the glazes of sherds found at Winchester and Bergen have low tin contents. It appears therefore, that, although the Winchester and Bergen samples are visually similar to those from Laverstock, they are unlikely to have originated from the Laverstock kilns. Of those we have examined the only English glaze with a comparable low tin content is that from the Tyler Hill kiln[8] near Canterbury.

One group of English glazes and one group of Continental glazes contained no detectable tin. This pottery, with the exception of a sample from Oslo, is of an earlier date (eleventh–twelfth century A.D.) than the remainder (late thirteenth–fourteenth century). The English group comprised three samples of 'Stamford ware', two from excavations at Thetford and one from Peveril Castle, Derbyshire. The Continental group comprised four samples of 'Andenne ware', one from an Andenne kiln, one from Antwerp, one from London and one from Canterbury; and the sample from Oslo designated on archæological grounds as 'Nottingham ware' (Dunning, G. C., private communication. Mr. Dunning suggests on typological grounds that this sample came from a kiln in that part of eastern England in the area Nottingham–Scarborough–King's Lynn). This spectroscopic similarity between the glazes of Stamford and Andenne wares is a striking confirmation of the theory put forward by Dunning[9] that the two wares developed from a common origin. It is clear, however, from observed differences in the composition of the fabric materials that these samples could not have originated from the same kiln.

Although all the Laverstock glazes had high tin contents, the relative amounts of tin and lead in the glaze varied significantly. On calculating the ratio of the observed intensities of lead and tin lines in the glaze, this was found to vary from 1·1 to 4·7. Placing the ratios in numerical order arranges the kilns from which the glazes originated in an order very close to the chronological order assigned to these kilns on the basis of archæological evidence, the lowest tin content arising from the earliest kiln. It is difficult to envisage any reasons why the tin content of the glazes should have been gradually increased, and it may well be that analyses on further samples will prove that this apparent systematic variation of the lead/tin ratio is fortuitous.

Two further points of interest have been noted from the examination of the Laverstock glazes. In all the kiln samples the calcium content of the glaze is higher than that of the fabric, suggesting that a calcium compound has been deliberately added, possibly to improve the adhesive properties of the glaze. Some of the samples examined had an additional stripe decoration in a darker colour than the body glaze. Comparative analyses of the two different coloured glazes from the same pot have revealed that this colour is solely due to the presence of enhanced concentrations of iron.

[1] Musty, J. W. G., *Mus. J.*, **60**, 251 (1961).
[2] Herteig, A. E., *Med. Arch.*, **3**, 177 (1959).
[3] Excavated by J. W. G. Musty. For interim reports see *Med. Arch.*, **2**, 213 (1958); *Med. Arch.*, **3**, 326 (1959).
[4] Borremans, R., and Lassance, W., *Archæologica Belgica*, **32** (1956).
[5] Stone, J. F. S., and Thomas, L. C., *Proc. Prehist. Soc.*, **22**, 37 (1956).
[6] Jope, E. M., *Trans. Bristol and Glos. Arch. Soc.*, **71**, 93 (1952).
[7] Jope, E. M., and Threlfall, R. I., *Antiquaries J.*, **39**, 254, Table 4 (1959).
[8] Spillett, P. J., Stebbing, W. P. D., and Dunning, G. C., *Archæologia Cantiana*, **55**, 57 (1943).
[9] Dunning, G. C., *Med. Arch.*, **3**, 41 (1959).

CÆSIUM-137 AND STRONTIUM-90 IN PRECIPITATION, SOIL AND ANIMALS IN NORWAY

By T. HVINDEN and A. LILLEGRAVEN

Division of Physics, Norwegian Defence Research Establishment, Kjeller

FROM fall-out measurements in Norway it seems that in this country the variations from one group of animals to another in uptake of radioisotopes may be greater than the variations in amounts of fall-out from one district to another. The geographical variations in fall-out are indicated by the amounts of cæsium-137 measured in precipitation at twelve places in Norway. The concentration in the total yearly precipitation for the past 4 years has been:

	1957	1958	1959	1960	
Average value	5·7	8·9	15	3·2	$\mu\mu$c. cæsium-137/l. precipitation
Maximum ,,	7·2	11	18	4·4	,, ,,
Minimum ,,	2·7	6·9	9·8	2·6	,, ,,

The yearly fall-out was:

	1957	1958	1959	1960	
Average value	6·1	7·6	11	2·3	mc. cæsium-137/km.^{2}
Maximum ,,	17	15	25	4·8	,, ,,
Minimum ,,	2·2	1·9	3·5	0·4	,, ,,

XUM

It appears that the maximum yearly cæsium-137 fall-out measured is approximately ten times the minimum. The cæsium-137 fall-out in 1960 was considerably lower than in the three foregoing years. The cumulative cæsium-137 fall-out at Bergen (60° 23′ N., 5° 21′ E.) from September 1956 to June 1961 is shown in two-monthly periods by curve *A* in Fig. 1. The total deposition during this period is about 70 mc./km.². The relatively heavy cæsium-137 fall-out during the period September–October 1957 may be due chiefly to the Windscale accident. Strontium-90 in precipitation at Bodø (67° 17′ N., 14° 24′ E.), and Tromsø (69° 40′ N., 18° 57′ E.) has been measured by the Atomic Energy Research Establishment, Harwell, as part of a world-wide precipitation sampling programme[1]. The concentration in the total yearly precipitation for the past 3 years has been:

	1958	1959	1960	
Bodø	6·2	17	3·8	μμc. strontium-90/l. precipitation
Tromsø	5·4	14	2·5	,, ,,

The yearly fall-out was:

	1958	1959	1960	
Bodø	7·1	18	2·6	mc. strontium-90/km.²
Tromsø	4·2	17	2·2	,, ,,

The cumulative strontium-90 fall-out at Bodø from April 1957 to December 1960 is shown in three-monthly periods by curve *C* in Fig. 1.

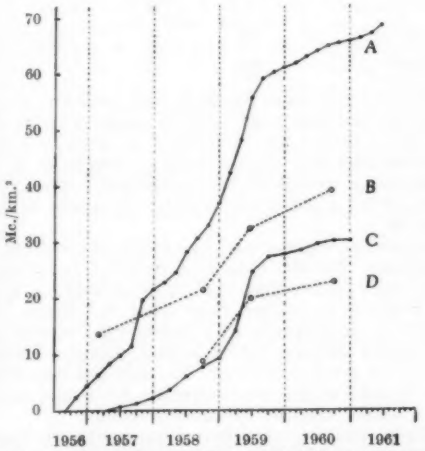


Fig. 1. *A*, Bergen: cæsium-137 in precipitation; *B*, Bergen: strontium-90 in soil; *C*, Bodø: strontium-90 in precipitation; *D*, Bodø: strontium-90 in soil

Cæsium-137 in soil was measured at several places in Norway in September 1960. Fig. 2 *A* shows the results plotted versus precipitation during the period from January 1, 1953, to the date of sampling. It appears that fall-out/mm. precipitation decreases with increasing precipitation. As indicated in Fig. 2, this may be due to an amount of dry fall-out equal to the fall-out deposited by a yearly precipitation of approximately 400 mm. It follows from this that in Norway the dry fall-out may be about 30 per cent of the total fall-out, the mean annual precipitation in Norway being about 900 mm. This is in reasonably fair agreement with what has been found for Kjeller[2]. The total cæsium-137 fall-out in Norway, which has an area of 324,000 km.², may be estimated from Fig. 2 *A* to be about 12,000 c. at the end of September 1960.

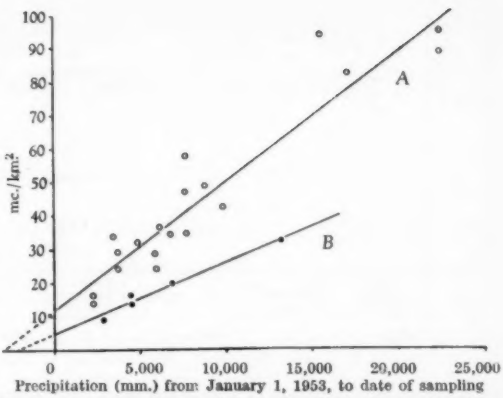


Fig. 2. *A*, cæsium-137 in soil, September 1960; *B*, strontium-90 in soil, June 1959

Strontium-90 in soil has been measured at several places in Norway by L. T. Alexander, as part of a world-wide soil-sampling programme[3]. The results for Bergen are shown by curve *B* in Fig. 1, and the results for Bodø by curve *D*. It appears from Fig. 1 that the strontium-90 fall-out at Bodø as deduced from soil measurements is lower than that measured in precipitation. The ratio is:

	Oct. 1958 to June 1959	July 1959 to Sept. 1960	Oct. 1958 to Sept. 1960
Strontium-90 in soil / Strontium-90 in precipitation	0·65	0·88	0·71

The difference might indicate leaching out of the soil or re-suspension of deposited fall-out, but more probably it is an indication of the difficulty involved in obtaining a representative value of the amount of precipitation falling on the ground, especially where snow-drifting occurs during winter. The differences in the sampling and measuring methods may also be important factors. The results for Bergen, shown in Fig. 1, give the following ratio of cæsium-137 in precipitation to strontium-90 in soil:

	March 1957 to Sept. 1958	Oct. 1958 to June 1959	July 1959 to Sept. 1960
Cæsium-137 in precipitation / Strontium-90 in soil	3·3	2·0	1·6

The high ratio during the period March 1957–September 1958 seems to agree with the suggestion that the cæsium-137 fall-out during the period September–October 1957 was due chiefly to the Windscale accident, the strontium-90 release of which has been given as less than 1 per cent of the cæsium-137 release[4]. If the cæsium-137 fall-out during this period is excluded, the ratio will be 2·2 instead of 3·3. In view of the different methods of sampling and measurements, the ratio of cæsium-137 to strontium-90 of about 2 seems to indicate that the soil data are in reasonably good agreement with the precipitation data. The measurements by the Atomic Energy Research Establishment, Harwell, give an average ratio of cæsium-137 to strontium-90 in fall-out nearer 1·7 (ref. 1).

Fig. 2 *B* shows strontium-90 in soil-samples collected during June 1959 plotted versus precipitation during the period January 1, 1953, to the date of sampling. In good agreement with the cæsium-137 results in Fig. 2 *A*, the strontium-90 results indicate an amount of dry fall-out equal to the fall-out deposited

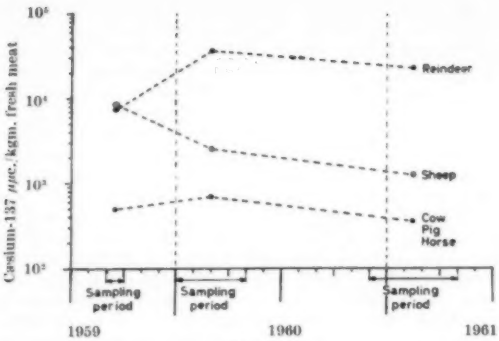


Fig. 3

by a yearly precipitation of about 300 mm. The total strontium-90 fall-out in Norway may be estimated from Fig. 2 *B* to be approximately 5,500 c. at the end of June 1959, of which about 30 per cent may be dry fall-out. Adding 4·9 mc./km.[2] found as the increment for Bodø from June 1959 to September 1960 (Fig. 1 *D*, the mean annual precipitation at Bodø being near the average for Norway), the total strontium-90 fall-out at the end of September 1960 may be estimated to be about 7,000 c. The ratio of cæsium-137 in soil to strontium-90 in soil, both as estimated, is about 1·7.

Cæsium-137 in meat was measured during 1959, 1960 and 1961. The results are:

	1959	1960	1961	
Cow, pig, horse	2 samples	31 samples	38 samples	
Average value	500	700	400	μμc. cæsium-137/kgm. fresh meat
Maximum ,,	600	2,800	1,500	,, ,,
Minimum ,,	300	200	~0	,, ,,
Sheep	2 samples	12 samples	9 samples	
Average value	8,000	2,500	1,200	μμc. cæsium-137/kgm. fresh meat
Maximum ,,	11,000	9,000	3,000	,, ,,
Minimum ,,	5,000	50	100	,, ,,
Reindeer	2 samples	6 samples	48 samples	
Average value	7,700	35,000	22,000	μμc. cæsium-137/kgm. fresh meat
Maximum ,,	9,300	56,000	37,000	,, ,,
Minimum ,,	6,200	16,000	7,000	,, ,,

The results are plotted in Fig. 3. The level found in the fall of 1959 is uncertain due to the very limited number of samples measured. During 1960–61 there appears to be a decrease of about 50 per cent in cæsium-137 concentration in all three groups of animals. It appears that, on the average, the uptake of cæsium-137 by sheep is about five times higher than by cow, pig and horse. The uptake by reindeer is about ten times higher than by sheep. As this difference is apparently not due to a difference in fall-out amounts, it seems reasonable to relate it to different grazing habits and different grazing field qualities. Within each group of animals there is a difference from district to district that may be related partly to differences in amounts of fall-out and partly to differences in conditions of grazing. There does not appear, however, to be any large difference in concentration of cæsium-137 in reindeer from southern Norway and northern Norway. The samples collected in 1961 were also analysed for potassium. The average values are:

Reindeer	4·4 gm. potassium/kgm. fresh meat	
Sheep	3·2 ,, ,,	,,
Cow, pig, horse	3·0 ,, ,,	,,

It appears that the potassium content in reindeer is about 40 per cent higher than in sheep, cows, pigs and horses.

Strontium-90 in animal bones from Norway has been measured by HASL, United States Atomic Energy Commission, New York Operations Office. The results are:

	1954 summer	1956 autumn	1958 autumn	
Cow	2 samples			
Average value	0·5			μμc. strontium-90/gm. calcium
Maximum ,,	0·8			,,
Minimum ,,	0·2			,,
Sheep	5 samples	14 samples		
Average value	4	17		μμc. strontium-90/gm. calcium
Maximum ,,	7	34		,,
Minimum ,,	2	6		,,
Reindeer		4 samples	10 samples	
Average value		100	150	μμc. strontium-90/gm. calcium
Maximum ,,		120	250	,,
Minimum ,,		40	110	,,

The four samples of reindeer bones collected in 1956 were analysed at the Atomic Energy Research Establishment, Harwell. The reindeer bones collected in 1958 were also analysed for strontium-89. The results are:

	Northern Norway 4 samples	Southern Norway 6 samples	
Average value	610	40	μμc. strontium-89/gm. calcium
Maximum ,,	650	94	,,
Minimum ,,	540	17	,,

The reindeer from southern and northern Norway were of comparable age and the samples collected at the same time. The difference in concentration of strontium-89 may then indicate a difference in time of arrival of strontium-89, or different grazing conditions giving a difference in delay of uptake of strontium-89.

In good agreement with the results for cæsium-137, it appears that the uptake of radioactive strontium by sheep is higher than that by cows, and the uptake by reindeer is higher than that by sheep. The relatively high uptake by reindeer may be related to the special grazing habits of this animal. Reindeer feed to a great extent on the slow-growing lichen. The uptake of fall-out materials by lichen appears to be higher than by other vegetation.

Three samples of lichen from northern Norway were measured during autumn, 1959. The results are:

Sample	μμc. cæsium-137/kgm. lichen	mc. cæsium-137/km.[2]
I	42,000	74
II	37,000	56
III	31,000	66
Average	36,000	65

From the yearly precipitation at the sampling place, the total amount of cæsium-137 deposited may be estimated from Fig. 2 to be about 60 mc./km.[2] at the end of 1959. It appears therefore that the efficiency of fall-out retention for lichen is very high.

Summarizing, it seems that for internal radiation from fall-out materials the variations in uptake of radioisotopes by animals may be more important than the uneven geographical distribution of fall-out.

[1] Crooks, R. N., *et al.*, Atomic Energy Research Establishment Report *R* 3349 (1960).
[2] Small, S. H., *Tellus*, **12**, No. 3 (1960).
[3] Alexander, L. T., *et al.*, United States Atomic Energy Commission Report *TID*-6567 (1961).
[4] Medical Research Council, *The Hazards to Man of Nuclear and Allied Radiations* (H.M.S.O., London, 1960).

A WORKING MODEL FOR THE UPPER ATMOSPHERE

By LUIGI G. JACCHIA

Smithsonian Institution Astrophysical Observatory, Cambridge, Mass.

NICOLET[1] has recently published a tabulation of atmospheric densities at altitudes of 150–2,000 km., computed from a model based on diffusion equilibrium with known boundary conditions at 120 km. and with helium diffusion starting at 105 km. The tabulation is given for 12 asymptotic temperature values, ranging from 773° K. to 2,133° K. Assuming the validity of such a model, it is possible to compute upper-atmospheric temperatures directly from densities derived from satellite accelerations, and—since the variation of the density is related to the variation of the decimetric solar flux[2,3]—it appears possible to establish a correspondence between the solar flux and the asymptotic temperature of the upper atmosphere.

For a specific value of the solar flux the temperature in the upper atmosphere ranges from a maximum at the centre of the diurnal bulge[3] to a minimum in the dark hemisphere. Unless we know accurately how the temperature varies with the location of a point in the atmosphere with respect to the bulge, we cannot reduce the temperatures derived from satellite accelerations to a standard location. Since, however, the variation of temperature appears to be quite small within a radius of at least 50° around the anti-bulge point[3], that is, the point 180° from the centre of the bulge, we can take satellite accelerations obtained within that region and establish for them a correspondence between solar flux and temperature. Results from four satellites are plotted in Fig. 1. The good inner agreement of the individual points, irrespective of satellite height, shows that Nicolet's model appears quite satisfactory. The relation between the minimum night-time temperature T_0 and the solar flux F at 10·7 cm. appears to be linear within the observed accuracy; the straight line in the diagram corresponds to the equation:

$$T_0 = 555° + 3°\ F \qquad (1)$$

where T_0 is expressed in °K. and F in units of 10^{-22} W./m.²/cycle/sec.

The accelerations of the four satellites from which this relation was derived extend over the interval February 1958–March 1961, during which the 10·7-cm. flux varied from a maximum close to 350 F units to a minimum of about 90, covering about 90 per cent of the expected range during the present 11-year solar cycle (the 1954 minimum was about 70, and it can be confidently expected that a similar reading will prevail in 1964–65). Atmospheric densities in the bulge can be represented with Nicolet's model, assuming that the temperature T_M at the centre of the bulge is in a constant ratio:

$$T_M = 1{\cdot}35\ T_0 \qquad (2)$$

to the night-time temperature T_0.

The decay of the temperature from the centre of the bulge outward will require a more refined analysis which is not yet completed. In my 1960 model, I (ref. 3) assumed that the density at a given height varied as $\cos^6 \frac{\psi'}{2}$, where ψ' is the angular distance from the centre of the bulge. On the basis of Nicolet's model it appears that for height under 700 km. the bulge must become sharper with increasing height, and this is corroborated by the comparison of recently obtained accelerations of satellites 1958 α (perigee height 350 km.) and 1958 β2 (perigee height 600 km.). In addition, the shape of the bulge must vary with the solar flux; only an analysis of temperatures through the bulge seems likely to yield satisfactory results.

In my 1960 atmospheric model[3] it was assumed that the atmospheric density ρ for any given height and any given ψ' is proportional to F^m; for simplicity it was assumed that m = constant = 1. Recently, Priester[4] found that m increases with height and is larger in the night-time atmosphere than in the sunlit hemisphere. It is interesting to note that this result can be deduced directly from Nicolet's model, if we assume that the relation between F and T_0 is given by equation (1). Calling ρ_0 the night-time density at a given height in the atmosphere, we shall have:

$$m_0 = \frac{\mathrm{d} \log \rho_0}{\mathrm{d} \log F} = \frac{T_0 - 555}{T_0} \frac{\mathrm{d} \log \rho_0}{\mathrm{d} \log T} \qquad (3)$$

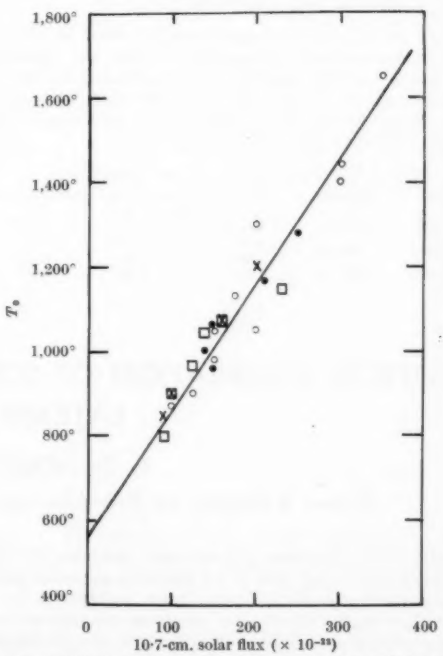


Fig. 1. Relation between the 10·7-cm. solar flux and the night-time temperature of the upper atmosphere, derived from four satellites using Nicolet's atmospheric model
●, 1958 β2; ×, 1959 α1; □, 1959 η; ○, 1958 α

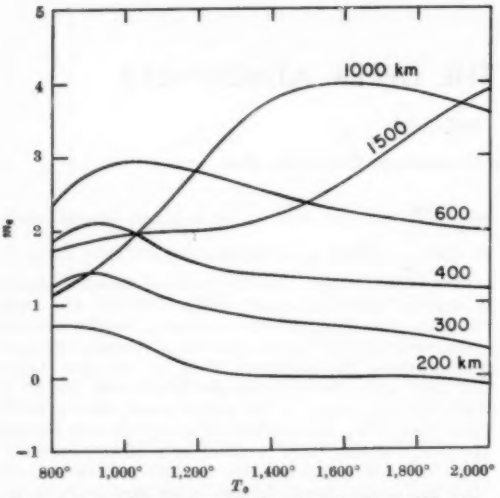


Fig. 2. Curves showing the variation of m_0 ($= \mathrm{d} \log \rho_0 / \mathrm{d} \log F$) with temperature in the night-time atmosphere for six representative heights

Curves of m_0 for six representative heights are shown in Fig. 2. The increase of m_0 with height is evident in the range 200–600 km.; at greater heights the picture is distorted by the increasing predominance of helium.

The curves of Fig. 2 are valid only for the anti-bulge point. For any other point, where the temperature is $T = \alpha T_0$ and the density at a given height is ρ:

$$m = \frac{\mathrm{d} \log \rho}{\mathrm{d} \log F} = \frac{T - 555\alpha}{T - 555}\, m_0 \qquad (4)$$

Since $\alpha > 1$, m is always smaller than m_0 for a given temperature. Considering that the general tendency of m_0 is to decrease with increasing temperature, we can easily see that the parameter m is considerably smaller in the diurnal bulge, compared with its value in the night-time atmosphere.

Table 1. Observed and Computed Values of m

Height	Satellite	Time	Observed m	Calculated m
350 km.	1958 α	Day	0·75 ± 0·2	0·6
		Night	1·05 ± 0·2	1·1
650 km.	1958 β2	Day	1·2 ± 0·3	1·2
		Night	3·0 ± 0·4	3·0
1,300 km.	1960 ι1	Day	2·0 ± 0·4	2·2

Priester's observed values of m are predicted almost exactly by Nicolet's model and equation (1), as can be seen from Table 1.

Lastly, Nicolet's model can correctly predict the observed increases of atmospheric density which accompany magnetic storms. As was pointed out by me[5,6], these atmospheric perturbations are nearly independent of geomagnetic latitude and occur simultaneously all over the globe. Accurate accelerations, as yet unpublished, computed by me in collaboration with Mr. J. Slowey, show that magnetic storms of comparable intensity give rise to larger drag perturbations when the background drag of the satellite is lower, either because of lower solar flux or because of greater angular distance of the perigee from the diurnal bulge. This is what would occur in Nicolet's model atmosphere if as a consequence of the corpuscular storm the temperature of the upper atmosphere were raised by the same amount all over the globe. A fair quantitative representation of all the observed atmospheric perturbations can be given by assuming that the temperature of the upper atmosphere is augmented by an amount ΔT proportional to the geomagnetic planetary index a_p, according to the relation:

$$\Delta T = 1{\cdot}5^\circ\ a_p \qquad (5)$$

This equation represents quite well also a number of smaller atmospheric perturbations in March and April 1961, revealed by the ultra-sensitive satellite 1961 δ1 (the 12-ft. balloon satellite).

Equations (1), (2) and (5) provide the means for computing temperatures of the upper atmosphere, for which Nicolet's model gives all other parameters. All three equations are only rough outlines of the true relations between the respective variables and should be susceptible of considerable refinements in the future; also, other effects on the atmospheric temperature should be revealed in the more detailed analysis which is now being made. It should be apparent, however, that Nicolet's model will provide an adequate basis for such an analysis, as had been expected by its author.

[1] Nicolet, M., *Smithson. Astrophys. Obs., Spec. Rep.* 75 (1961).
[2] Priester, W., and Martin, H. A., *Mitt. Univ. Sternwarte Bonn*, No. 29 (1960).
[3] Jacchia, L. G., *J. Geophys. Res.*, **65**, 2775 (1960).
[4] Priester, W., *J. Geophys. Res.* (in the press).
[5] Jacchia, L. G., *Nature*, **183**, 1662 (1959).
[6] Jacchia, L. G., *Smithson. Astrophys. Obs., Spec. Rep.* 62 (1961).

OPTICAL CALIBRATION OF SOME MONODISPERSE POLYSTYRENE LATEXES IN ARRAYS

By Dr. HERBERT E. KUBITSCHEK

Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois

THE monodisperse polystyrene latexes, which have been used widely by electron microscopists as secondary standards, have been calibrated in a variety of ways, chiefly by electron microscopy of single particles[1] and light scattering from suspensions[2]. These methods are tedious, and their results have afforded little or no applicability to optical microscopy. It seemed possible that arrangements of spherical monodisperse particles in arrays might permit rapid and precise determination of their mean diameters, since a number of particles could be included in a single measurement.

The existence of an array of particles, as in Fig. 1*d*, is evidence for their uniformity, their contiguity, and the linearity of their rows. For microscopic particles, the greatest advantage of measuring particles in a

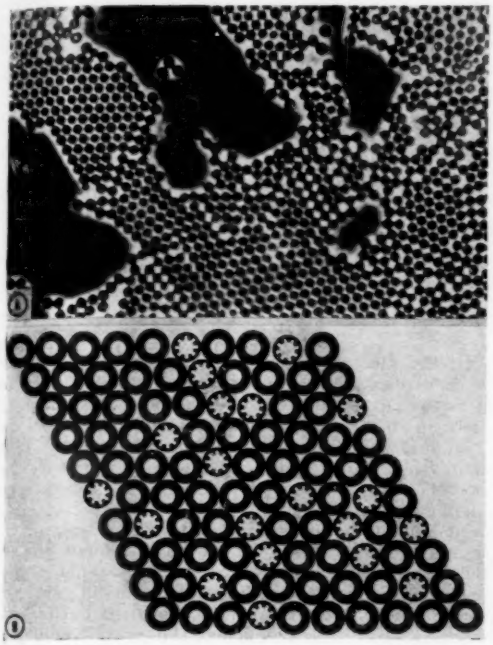


Fig. 1. *A*, arrays of 1·15μ polystyrene particles in oil. *B*, a standard two-component washer array

row is that errors arising from diffraction can be made negligible by choosing the line of symmetry between the diffraction images of the particles, or alternatively, measuring between the centres of two particles. The contribution of error of measurement is further reduced, of course, by spanning a number of particles in the row in a single measurement; variations in particle diameter tend to cancel one another, usually giving an improved estimate of the mean diameter. However, the cancellation is imperfect since a small particle may be only loosely encircled by its neighbours. Thus, a measurement which spans one of these small particles will give a result which over-estimates the true mean.

Simple models indicate that the over-estimate $\Delta\bar{x}$ of the mean diameter should be proportional to the standard deviation σ of the measured distribution of apparent particle diameters:

$$\Delta\bar{x} = k\,\sigma, \quad k = \text{constant} \qquad (1)$$

Thus, a knowledge of the value of k will allow an estimate, D, of the true mean diameter from the apparent mean diameter $\bar{x}$; that is, $D = \bar{x} - \Delta\bar{x}$. It should be possible to calculate k for the triangular arrays shown in Fig. 1, but to my knowledge this problem is unsolved.

The coefficient k was determined empirically from measurements with a vernier caliper of two-dimensional triangular arrays of machinists' washers of known diameters arranged in the rhombic pattern shown in Fig. 1*B*. Two-component arrays, as in Fig. 1*B*, were formed of eighty $\frac{1}{4}$-in. diameter washers with twenty slightly smaller washers added at random locations. The twenty rows of ten washers parallel to the edges of the rhombus were measured to obtain the apparent mean diameter $\bar{x}$, which was then compared with the known true mean to give $\Delta\bar{x}$. Fig. 2 displays the values obtained in this manner for $\Delta\bar{x}/\bar{x}$ and the corresponding values for $\sigma/\bar{x}$ for several two-component arrays, for one array constructed of three sizes of washers (three-component) and for six arrays formed from washers especially constructed to have a normal distribution of diameters (coefficient of variation = 2·36 per cent). These (array sample) values have been fitted with a straight line by least mean squares; the data are consistent with equation (1) giving $k = 0{\cdot}461$ (S.E., 0·029). This value for k is directly applicable to the triangular arrays of spherical polystyrene particles even though these particles probably are tangent to a glass surface, removing their centres from a common plane. In this case the mean diameter is under-estimated approximately by the fraction $\frac{1}{4}(\sigma/\bar{x})^2$, which would have a value of about 0·01 per cent for the smallest three latexes in Table 1 according to the values of Bradford and Vanderhoff[1].

The formation of arrays from polystyrene latexes has been somewhat unpredictable. With the aid of a wire loop, a minute volume (~ 1 μl.) of a latex (kindly supplied by Dr. John Vanderhoff, Dow Chemical Co.) was added to a drop of microscope immersion oil (Cargille's; index of refraction, 1·515) on a microscope slide and mixed gently, and a coverslip was added with moderate pressure. Close-packed triangular arrays of spheres in single layers were found in some of these preparations. Measurements of these arrays were made with a phase microscope (× 970) having an ocular reticle calibrated to 0·3 per cent. The results of these measurements, summarized in Table 1, appeared independent of the number of particles in the array, with the possible exception of the 0·5μ particles for which arrays of more than twenty particles were not obtainable.

For each latex the apparent mean diameter was determined from:

$$\bar{x} = \frac{\Sigma L_i}{N} \qquad (2)$$

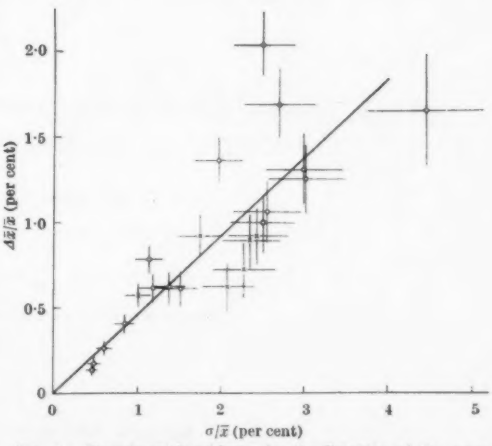


Fig. 2. Experimental evidence for the linearity of the over-estimate $\Delta\bar{x}$ as a function of the standard deviation σ in the measured mean diameter $\bar{x}$ of washers in arrays. The standard error in the values of $\Delta\bar{x}/\bar{x}$ is shown as the vertical line segment above or below each point and was computed from the usual standard error of the mean for the $n = 20$ measurements on each array. The corresponding horizontal line segments at either side of each point represent the estimated standard deviation of σ. The slope of the least mean square line is $k = 0{\cdot}461$ (S.E., 0·029). ○, Two-component distribution; □, three-component distribution; ×, normal distribution. S.E. $_{\Delta\bar{x}} \simeq \frac{\sigma}{\sqrt{n}}$; $_{\sigma\sigma} \approx \frac{\sigma}{\sqrt{2n}}$

Table 1. Apparent Mean Diameters ($\bar{x}$) and Estimated Mean Diameters (D) from Optical Measurements of Monodisperse Polystyrene Latexes

	Latex number				
	15*N*-8	*LS*-066-*A*	*LS*-067-*A*	*L*-3830-1	*L*-3830-19-*B*
N, total No. of particles	100	640	599	78	57
R, No. of rows	16	39	51	8	9
$\bar{x} \pm$ S.E.$_{\bar{x}}$, mμ	530 ± 4	804 ± 2	1,165 ± 2	1,721 ± 4	3,245 ± 3
$\Delta\bar{x}$, over-estimate, in per cent of $\bar{x}$	3·25	2·63	1·87	0·82	0·35
$D \pm$ S.E.$_D$,* mμ	512 ± 6	783 ± 5	1,143 ± 6	1,710 ± 7	3,234 ± 11
Previous measurements					
D† ± S.E.$_D$, mμ	511 ± 6·4	814 ± 6·4	1,171 ± 9·7		
D‡ ± S.E.$_D$, mμ	511 ± 6·6	826 ± 22·6	1,166 ± 28·2		

* The 0·3 per cent standard error in the absolute calibration of the microscope stage has been included.
† Data of Bradford and Vanderhoff (ref. 1).
‡ Data of Bateman *et al.* (ref. 2).

where L_i is the measured length of the n_i particles in row i and N is the total number of particles which are measured, the sum being taken over all rows R. The standard error of the mean, S.E.$_{\bar{x}}$, is estimated by:

$$(\text{S.E.}_{\bar{x}})^2 = \frac{\Sigma \frac{1}{n_i} (L_i - n_i\bar{x})^2}{N(r-1)} \qquad (3)$$

where r is the number of different row-lengths measured. The standard deviation σ_x of the values for apparent particle diameter is given by:

$$\sigma_x^2 = N(\text{S.E.}_x)^2 \qquad (4)$$

For each latex, the error of measurement was estimated independently from repeated measurements on a pair of adjacent particles, and found to be about 80 mμ, although increasing slightly for the largest and smallest particles. These errors are about an order of magnitude greater than those obtained for the mean diameters, demonstrating the efficiency of the array method.

The array method leads to significantly lower values for the 0·8μ and the 1·1μ particles when compared with the electron microscope values[1]. This might be due to an increase in cross-sectional area because of flattening of the particles in the electron beam; such increases are observed for larger particles (Vanderhoff, J. W., personal communication). It is likely that refinement of this method through the use of photographic enlargements will yield even smaller mean diameters for these particles, since fault-lines now indiscernible may be recognized. It should be noted also that the measurement of particles in arrays leads to stratified sampling, disregarding the rare, very large or very small particles; thus, minor components of the distribution are rejected. But, in consequence, the reproducibility of this method is greater than would be expected under random sampling. The array method is, in principle, extendable to investigations of the variation of spherical viruses and macromolecules in crystalline arrays.

This work was performed under the auspices of the U.S. Atomic Energy Commission.

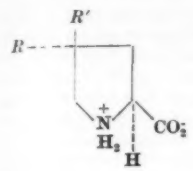
[1] Bradford, E. B., and Vanderhoff, J. W., *J. Appl. Phys.*, **26**, 864 (1955).
[2] Bateman, J. B., Weneck, E. J., and Eschler, D. C., *J. Colloid Sci.* **14**, 308 (1959).

STRUCTURES AND CONFORMATIONS OF SOME NATURALLY OCCURRING DERIVATIVES OF PROLINE

By R. J. ABRAHAM and K. A. McLAUCHLAN
Basic Physics Division, National Physical Laboratory
SHIRLEY DALBY, Prof. G. W. KENNER and R. C. SHEPPARD
Department of Organic Chemistry, University of Liverpool
AND
L. F. BURROUGHS
Agricultural Research Station, Long Ashton, Bristol

THE importance of proton magnetic resonance spectroscopy as an aid to the determination of the molecular structures of organic compounds is now well established. One purpose of this article is to show that, in addition, this technique may well prove to be of equal value in the determination of the conformations of molecules in solution.

The compounds examined in this investigation were all C_4 substituted derivatives of proline, namely, *cis* (allo) (I) and *trans*-hydroxyproline (II), *cis* (III) and *trans*-4-methylproline (IV), and a naturally occurring proline derivative for which the alternative structures 4-hydroxymethyl (V) or 4-hydroxy-4-methylproline (VI) have been suggested[1–5].



I	R=H, R'=OH
II	R=OH, R'=H
III	R=H, R'=Me
IV	R=Me, R'=H
V	R,R'=H,CH_2OH
VI	R,R'=OH,Me

(The synthesis of *trans*-4-methyl-L-proline, which is identical with the natural amino-acid from apple and pear juices, has already been described[1]. A modification of this synthesis has now yielded *cis*-4-methyl-D-proline, enantiomeric with the amino-acid previously isolated from an antibiotic[2]. Full details of this synthetic work will be submitted for publication in the *Journal of the Chemical Society*.)

All the amino-acids were examined as zwitterions in deuterium oxide solution on a Varian spectrometer operating at 60 Mc./s., using dioxan as an internal reference (τ = 6·43). The results are summarized in Table 1 with the nomenclature that *a* and *b* refer to protons on the opposite and the same side respectively of the ring as the CO_2^- group.

The compounds all give complex spectra, some of which have not been fully analysed. The coupling constants obtained from these latter spectra are simply first-order values, that is, they are the actual spacings between particular peaks in the spectrum. Such an analysis only gives approximate values of these coupling constants, which may be in error by as much as 1 c.p.s. in some cases. However, the spectra of the two hydroxy-L-prolines were completely analysed[6], to give all the coupling constants between adjacent protons around the ring. The values of these coupling constants vary from 1 to 10 c.p.s. (Table 1). Both these compounds show, in addition, a long-range coupling over four bonds, between one 3-proton and one 5-proton. The variation of adjacent coupling constants has been explained[7] by means of equation (1):

$$J = K_1 \cos^2\varphi + K_2 \qquad (1)$$

relating the coupling constant J to the dihedral angle φ between the relevant C—H bonds, which in turn depends on the precise conformation of the molecule in solution. (The factor K_2, which was predicted to be very small, will be ignored henceforth.)

In each of the two compounds we have three pairs of coupling constants J_{2-3}'s, J_{3-4}'s and J_{4-5}'s. Each pair, using (1), will give the K_1 and φ for this particular fragment. Only two φ's are necessary to determine the conformation of the molecule. The value of the third pair of couplings thus serves as a test of the applicability of the Karplus equation (1) to the molecule considered.

In the case of the *trans* hydroxyproline the conformation shown in Fig. 1 satisfactorily explains all the observed constants for an angle of buckle of 50–60°. This is an envelope conformation, analogous to the C_s form of cyclopentane[8]. The Karplus equation (1) was derived assuming sp^3 hybridization of the carbon atoms concerned. For the above angle of buckle, the C—C—C angles decreased to around 100°. Thus both equation (1) and the angle of buckle may well be only approximate in this case. However, these results certainly suggest that the molecule exists in solution in a single conformation which is considerably more buckled than has been realized previously.

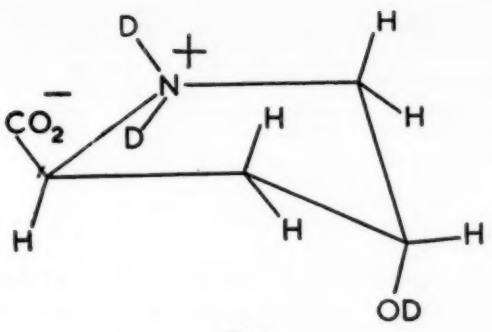


Fig. 1

In the conformation suggested here the OH group attains a pseudo axial configuration while the larger CO_2^- group attains a pseudo equatorial position. (The molecular plane should be considered more correctly as defined by centres 1.2 and 5 than by centres 1.2.3. and 5 (ref. 8).) Presumably the influence of the larger substituent group is the dominant factor.

It is of interest to note that the conformation of the molecule in solution differs from that in the solid, in which a similar type of conformation is found but the angle of buckle is now only 20° (ref. 9).

In the case of the *cis* (allo) hydroxyproline, we now find that there is no single conformation which will predict, by applying equation (1), the observed coupling constants. One possible explanation of this is that the molecule exists in more than one conformation in solution. These conformers would be interchanging rapidly and the observed J's would then be averages of the J's of the particular conformers. Two factors tend to oppose this. Such an averaging process would normally tend to equalize all the coupling constants. Secondly, the long-range coupling would be either averaged out or found on both C_2 and C_5 protons. None of these predictions is observed.

One other possibility is that the Karplus equation (1) does not hold for this molecule, for example, a strong hydrogen bond between the OH and carboxyl groups may distort the C—C—C angles even more than in the *trans* hydroxyproline, in which case the Karplus equation may no longer apply. The formation of an internal lactone in derivatives of allohydroxyproline[10] does suggest that the ring can be considerably deformed.

Whatever the true reason for this discrepancy, two factors emerge from this investigation. First, the observed J's in the allohydroxyproline molecule strongly suggest that the molecule exists in solution in a considerably buckled conformation, or conformations. Secondly, the Karplus equation can give in certain cases information on the precise conformation of molecules in solution, which is extremely difficult to obtain by any other technique; but the results obtained by the application of this equation to other molecules, particularly those which may be expected to occur in a highly strained form, must be considered with some caution.

Although the remaining spectra considered here are too complex for complete analysis, they still provide some further information. In particular, the spectrum (Fig. 2*a*) of the naturally occurring proline derivative (V) or (VI) allows an unambiguous decision in favour of the former, and considered in relation to

Table 1

Molecule	C_4 substituent	Proton chemical shifts (τ-values)						Proton coupling constants (c.p.s.)			
		2a	3a	3b	4b	5a	5b	2a–3a	2a–3b	4b–5a	4b–5b
L-Proline		6·0	—†	—†	—†	6·78	6·78	7·1*	7·1*	6·3*	6·3*
L-Hydroxyproline (II)	OH(4a)	5·83	7·77	8·05	5·52	6·83	6·69	7·66	10·44	1·22	4·09
Allohydroxy-L-proline (I)	OH(4b)	5·96	7·69	7·95		6·81	6·75	4a : 5·6	10·48	3·84	(4a) 4·57 / 0·94
Trans-4-methyl-proline (IV)	CH_3(4a)	5·98	—†	—†	—†	7·31	6·60	$4CH_3$: 9·06	4·4*	8·6*	8·3*
cis-4-Methylproline (III)	CH_3(4b)	6·00	8·53	—‡		7·19	6·61	$4CH_3$: 9·06 4a‡	9·0*	8·1*	8·6*
Hydroxymethyl-proline (V)	CH_2OH	5·99	8·37	—§	—§	6·98	6·66	$4CH_2OH$: 6·57	8·5*	8·5*	7·8*

Molecule	Proton coupling constants (c.p.s.)	
	3a–4a	3b–4a
L-Proline		
L-Hydroxyproline (II)	3a–3b : 14·06 3a–4b : 1·41 3b–4b : 4·31	5a–5b : 12·69 5a–5a : 1·28
Allohydroxy-L-proline (I)	3a–3b : 14·23 3a–4a : 4·71 3b–4a : 2·09	5a–5b : 12·50 3b–5b : 1·37
Trans-4-methyl-proline (IV)		5a–5b : 11·0*
cis-4-Methylproline (III)		5a–5b : 10·8*
Hydroxymethyl-proline (V)		5a–5b : 11·6*

* Denotes first order values. † Complex multiplet τ = 7·6–8·4. ‡ Complex multiplet τ = 7·4–7·9. § Complex multiplet τ = 7·8–7·9.

the spectra (Figs. 2*b* and *c*) of the *cis* and *trans*-4-methylprolines, provides an indication of its possible stereochemistry. (After this part of the work was complete, we were informed by Prof. F. C. Steward and K. Biemann that they had reached a similar conclusion by mass spectrometry[11]. Their results were considered to favour a *trans* configuration.) Thus the 4-hydroxy-4-methyl structure (VI) is immediately eliminated, since no methyl peak is found, and the spectrum agrees very well with the 4-hydroxymethyl alternative (V). In particular, the doublet of intensity two protons at 6·57 is characteristic of a —CH—CH_2—O— fragment, and the peaks at 5·99, 7·6 (approximately) and 8·37, and 6·66 and 6·98 fall in the expected ranges for the H_2, H_{3a} and H_{3b}, and H_{4a} and H_{5b} protons of the proline ring. Thus, all the main groups in the spectrum are consistent with the proposed formula, and define both the nature of the substituent and its position of attachment to the proline ring.

The spectrum of (V) (Fig. 2*a*) shows very marked similarities with that (Fig. 2*b*) of the *cis*-4-methylproline (III) in both the structure of the H_2 peak and the relative chemical shifts of the H_2 protons, which are quite different (Figs. 2*b* and *c*) for the two epimeric 4-methylprolines (III) and (IV). This may be considered some support for the assignment of a *cis* configuration to natural 4-hydroxymethylproline (V). (This result is unexpected since it is the *trans* isomer of 4-methylproline which occurs alongside 4-hydroxymethylproline in apple and pear juices.[1]) However, it should be emphasized that to confirm this statement

Fig. 2. Proton resonance spectra (60 Mc./s.) with integrals of (*a*) 4-hydroxymethylproline, (*b*) *cis*-4-methylproline and (*c*) *trans*-4-methylproline in deuterium oxide solution

it would be necessary to determine the exact reasons for the different spectra of the *cis* and *trans* isomers of 4-methylproline, which in turn requires a detailed analysis of these very complex spectra. Further research in this direction is in progress.

[1] Burroughs, L. F., Dalby, S., Kenner, G. W., and Sheppard, R. C., *Nature*, **189**, 394 (1961).
[2] Kenner, G. W., and Sheppard, R. C., *Nature*, **181**, 48 (1958).
[3] Hulme, A. C., *Nature*, **174**, 1055 (1954).
[4] Urbach, G., *Nature*, **175**, 170 (1955).
[5] Hulme, A. C., and Steward, F. C., *Nature*, **175**, 171 (1955).
[6] Abraham, R. J., and McLauchlan, K. A., *Mol. Phys.* (in the press).
[7] Karplus, M., *J. Chem. Phys.*, **30**, 11 (1959).
[8] Kilpatrick, J. E., Pitzer, K. S., and Spitzer, R., *J. Amer. Chem. Soc.*, **69**, 2483 (1947).
[9] Donohue, J., and Trueblood, K. N., *Acta Cryst.*, **5**, 419 (1952).
[10] Patchett, A. A., and Witkop, B., *J. Amer. Chem. Soc.*, **79**, 185 (1957).
[11] Biemann, K., Deffner, G. G. J., and Steward, F. C., *Nature*, **191**, 380 (1961).

RELATIONSHIP BETWEEN OXIDATION OF ISOCITRATE AND METABOLISM OF FATTY ACIDS IN RABBIT-HEART SARCOSOMES

By Dr. W. C. HÜLSMANN

Laboratory of Physiological Chemistry, University of Amsterdam

IN the presence of adenosine triphosphate (ATP) and [^{14}C]acetate, the oxidation of intermediates of the Krebs cycle by rabbit-heart sarcosomes (mitochondria) is linked with the incorporation of acetate into the mitochondrial long-chain fatty acids[1]. It has now been found that two closely related but separate mechanisms are involved, one associated with the oxidation of α-ketoglutarate, succinate or malate and the other with the oxidation of isocitrate. The two mechanisms are additive. The first is completely sensitive to antimycin and the second is insensitive. Since the first step of fatty acid synthesis is the condensation of malonyl-coenzyme A with acetyl-coenzyme A (ref. 2), it is thought likely that the Krebs-cycle intermediates provide β-keto carboxylic acids (oxalo-acetate for the first mechanism, oxalosuccinate for the second) for reaction with acetyl-coenzyme A to form malonyl-coenzyme A (cf. ref. 3).

Oxaloacetate + acetyl-coenzyme A ⇌ pyruvate + malonyl-coenzyme A (1)

Oxalosuccinate + acetyl-coenzyme A ⇌ α-ketoglutarate + malonyl-coenzyme A (2)

It is known that isolated heart sarcosomes oxidize added citrate or isocitrate very slowly[4,5]. During the investigation of the incorporation of acetate into long-chain fatty acids linked with the oxidation of isocitrate, it was found that the addition of acetate stimulated about six-fold the rate of uptake of oxygen with isocitrate as substrate, and that dinitrophenol abolished this stimulation. Measurements of the disappearance of the Krebs-cycle tricarboxylic acids [$-\Delta$(isocitrate + citrate + aconitate)] confirmed that these effects on uptake of oxygen reflected the oxidation of isocitrate.

Table 1 shows that a number of other compounds are also able to stimulate the oxidation of isocitrate, namely, β-hydroxybutyrate, α-ketoglutarate, succinate, malate, glutamate or diphosphopyridine nucleotide (DPN) in very high concentrations. The effects of α-ketoglutarate and β-hydroxybutyrate, of α-ketoglutarate and acetate, and of diphosphopyridine nucleotide and acetate were not additive. Dinitrophenol inhibited the effects of all these compounds, except that of diphosphopyridine nucleotide (Table 1 shows that the stimulation by diphosphopyridine nucleotide was the same in the presence and absence of dinitrophenol). Malonate inhibited the stimulation by acetate or β-hydroxybutyrate, but had little effect with α-ketoglutarate (the effect of malonate on the stimulation brought about by the other compounds was not tested).

The measurements shown in Table 1 were carried out under the same conditions as used in work on the incorporation of acetate into long-chain fatty acids[1], that is, in the presence of triphosphopyridine nucleotide (TPN), coenzyme A and adenosine triphosphate, and in the absence of hexokinase and glucose. In a reaction mixture commonly used for investigations of oxidative phosphorylation (that is, no added pyridine nucleotide or coenzyme A, adenosine diphosphate in place of the triphosphate and added hexokinase and glucose), neither acetate nor β-hydroxybutyrate had any appreciable effect on the disappearance of isocitrate. α-Ketoglutarate stimulated the disappearance substantially, and this stimulation was not affected by malonate. It appears, then, that adenosine triphosphate is necessary for the stimulation of the oxidation of isocitrate by acetate or β-hydroxybutyrate, but not by α-ketoglutarate.

It is known[6,7] that heart sarcosomes, previously depleted of pyridine nucleotide by incubation in phosphate, oxidize isocitrate in the presence of added pyridine nucleotide by the sequence:

Isocitrate + TPN^+ → α-ketoglutarate + CO_2 + H^+ + TPNH (3)

TPNH + DPN^+ ⇌ TPN^+ + DPNH (4)

DPNH + $\frac{1}{2}O_2$ + H^+ → DPN^+ + H_2O (5)

Klingenberg and Slenczka[8] have shown, however, that in rapidly respiring intact mitochondria the diphosphopyridine nucleotide is largely oxidized while the triphosphopyridine nucleotide is largely reduced, suggesting that the accessibility of intramitochondrial TPNH to DPN^+ to one another or to the pyridine nucleotide transhydrogenase is limited. This is probably the reason for the poor oxidation of isocitrate by isolated mitochondria.

The stimulation of the oxidation of isocitrate by diphosphopyridine nucleotide can be simply explained by assuming that high concentrations of this compound are able to break the accessibility barrier and

Table 1. Oxidation of Isocitrate by Rabbit-heart Sarcosomes

Substrate or co-factor added	Inhibitor added	− Δ(isocitrate + citrate + aconitate) (μmoles) Exp. 1	Exp. 2	Exps. 3 and 4
None	none	0·75	0·55	0·40
None	dinitrophenol	0·17	0·20	
Acetate	none	2·32	1·57	1·56
Acetate	dinitrophenol	0·68		0·12
Acetate	antimycin	0·18		0·12
Acetate	malonate			0·34
DL-β-Hydroxybutyrate	none	2·83	2·06	1·89
DL-β-Hydroxybutyrate	dinitrophenol	0·69		
DL-β-Hydroxybutyrate	antimycin			0·11
DL-β-Hydroxybutyrate	malonate			0·66
DPN⁺ (3 m*M*)	none	1·99		
DPN⁺ (3 m*M*)	dinitrophenol	1·40		
DPNase-treated DPN⁺ (3 m*M*)	dinitrophenol	0·29		
DPN⁺ (1 m*M*)	dinitrophenol	0·80		
α-Ketoglutarate	none		2·00	1·87
α-Ketoglutarate	dinitrophenol			0·82
α-Ketoglutarate	malonate			1·66
Succinate	none			1·69
Succinate	dinitrophenol			0·39
Malate	none			1·36
Malate	dinitrophenol			0·56
Glutamate	none			1·80

Sucrose, 50 m*M*; potassium chloride, 9·5 m*M*; ethylenediaminetetraacetate, 1 m*M*; potassium phosphate buffer (*p*H 7·5), 50 m*M*; magnesium chloride, 5 m*M*; triphosphopyridine nucleotide (TPN⁺), 0·2 m*M*; adenosine triphosphate (ATP), 10m*M*; L*s*(+)-isocitrate, 5 m*M*; coenzyme A, 0·18 m*M*; glutathione, 1·8 m*M*; rabbit-heart sarcosomes (isolated in sucrose-ATP, ref. 26), 1·72 mgm. protein in exp. 1, 1·62 mgm. in exp. 2, 1·72 mgm. in exp. 3 and 1·20 mgm. in exp. 4. Other additions, where shown: acetate, 3 m*M*; DL-β-hydroxybutyrate, 1 m*M* in exp. 1, 3 m*M* in exp. 2, 12 m*M* in exps. 3 and 4; α-ketoglutarate, 30 m*M*; succinate, 40 m*M*; L-malate, 30 m*M*; L-glutamate, 30 m*M*; 2,4-dinitrophenol, 0·1 m*M*; antimycin, 1 μgm./ml.; malonate, 10–12 m*M*. 2 per cent ethanol was also present in all flasks of exps. 1 and 2. The reaction was carried out in a shaking manometer flask with 10 per cent potassium hydroxide and a filter paper in the centre well. The reaction was stopped after 40 min. (exp. 1), 45 min. (exp. 2), 60 min. (exps. 3 and 4), at 25° by adding trichloroacetic acid (final conc. 5 per cent). Isocitrate + citrate + aconitate were determined on the neutralized supernatant by measuring the reduction of TPN⁺ in the presence of isocitrate dehydrogenase and aconitase. The values given for exps. 3 and 4 are the means of two experiments.

promote the rate-limiting reaction (4). It remains to be explained how acetate and the other compounds listed in Table 1 also promote the oxidation of intramitochondrial reduced triphosphopyridine nucleotide (TPNH). It is natural to link this process with the mechanism of fatty acid synthesis, which is known to involve the oxidation of TPNH (refs. 9–11). For example, it seems possible that acetoacetyl-coenzyme A could bring about the transhydrogenase reaction (4) by reactions (6) and (7):

TPNH + acetoacetyl-coenzyme A + H^+ → TPN⁺ + β-hydroxybutyryl-coenzyme A (6)

β-Hydroxybutyryl-coenzyme A + DPN⁺ → acetoacetyl-coenzyme A + DPNH + H^+ (7)

the sum of which is reaction (4). Experiments to be reported elsewhere have shown the presence of an enzyme catalysing the oxidation of TPNH by acetoacetyl-coenzyme A in digitonin extracts of rabbit-heart sarcosomes. Whether the product of reaction (6) has the L-configuration, which is the substrate for reaction (7) (ref. 12), is of minor importance, since mechanisms for converting the L-form to the D-form are known[13,14].

Acetate can yield acetoacetyl-coenzyme A by reactions (8) + (9) (refs. 15 and 16) or (8) + (10):

Acetate + coenzyme A + ATP → acetyl-coenzyme A + AMP + pyrophosphate (8)

Acetyl-coenzyme A + malonyl-coenzyme A → acetoacetyl-coenzyme A + coenzyme A + CO_2 (9)

2 acetyl-coenzyme A ⇌ acetoacetyl-coenzyme A + coenzyme A (10)

The malonyl-coenzyme A could be derived by reaction (2) from the oxalosuccinate formed by oxidation of isocitrate.

β-Hydroxybutyrate can be converted to acetoacetyl-coenzyme A by reactions (11) (ref. 12) and (12):

β-Hydroxybutyrate + coenzyme A + ATP ⇌ β-hydroxybutyryl-coenzyme A + AMP + pyrophosphate (11)

β-Hydroxybutyryl-coenzyme A + $\frac{1}{2}O_2$ → acetoacetyl-coenzyme A + H_2O (12)

Table 1 shows that the oxidation of isocitrate with added acetate or β-hydroxybutyrate is inhibited by dinitrophenol as would be expected according to these equations. The slow oxidation in the absence of acetate, which might be due to endogenous fatty acid, is also inhibited by dinitrophenol.

The stimulation of disappearance of isocitrate by succinate or malate in the absence of added acetate could be explained similarly, since these compounds give rise to acetyl-coenzyme A by oxidation of the pyruvate formed by decarboxylation of the oxaloacetate, and to malonyl-coenzyme A by reaction (1). Thus acetoacetyl-coenzyme A can be formed by reaction (9). The contribution of reaction (10) to the formation of acetoacetyl-coenzyme A in the absence of added acetate is likely to be small, because the equilibrium of this reaction is far to the left[15].

The stimulation of isocitrate oxidation by α-ketoglutarate cannot be explained on the same basis since it is not inhibited by malonate. It seems most likely that succinyl-coenzyme A formed by the oxidation of α-ketoglutarate is involved, but how is not certain. It is possible that acetoacetyl-coenzyme A is formed from traces of acetoacetate in the preparation by reaction (13) (refs. 17 and 18):

Succinyl-coenzyme A + acetoacetate ⇌ succinate + acetoacetyl-coenzyme A (13)

although no acetoacetate could be detected in the sarcosomal preparations by Walker's method[19]. The partial sensitivity to dinitrophenol of the stimulation of citrate oxidation by α-ketoglutarate might be due to the appreciable activity of the succinyl-coenzyme A deacylase in the presence of dinitrophenol and absence of fluoride[20]. Glutamate would be expected to act in the same way as α-ketoglutarate, since the oxidation of α-ketoglutarate to succinate is a compulsory step in the oxidation of this substrate by heart sarcosomes[21].

Two explanations for the effect of malonate might be considered: (i) by inhibiting succinic dehydrogenase it prevents the formation of oxaloacetate by the oxidation of isocitrate, with consequent decrease in the amount of acetoacetyl-coenzyme A formed from malonyl-coenzyme A by reaction (9); (ii) malonate causes the loss of acetyl-coenzyme A with formation of free acetoacetate by reaction (15), which is the sum of reactions (14) (ref. 22) and (9):

Malonate + acetoacetyl-coenzyme A ⇌ malonyl-coenzyme A + acetoacetate (14)

Malonyl-coenzyme A + acetyl-coenzyme A → acetoacetyl-coenzyme A + coenzyme A + CO_2 (9)

Malonate + acetyl-coenzyme A → acetoacetate + coenzyme A + CO_2 (15)

The lack of effect of malonate on α-ketoglutarate-stimulated isocitrate oxidation is in favour of the second possibility, since acetoacetate formed by reaction (15) could readily be converted to acetoacetyl-coenzyme A by reaction (13).

Thus, it is possible to explain the stimulations by all the substrates listed in Table 1 in terms of their ability to promote the formation of acetoacetyl-coenzyme A (or other keto-acyl-coenzyme A intermediates of fatty acid synthesis and breakdown). An initial lag was found both in the incorporation of acetate into fatty acid promoted by citrate[1] and in the acetate-promoted oxidation of isocitrate (for example, 0·42 μmole in the first 15 min., 1·25 μmoles in the second and 0·76 μmole in the third). This is to be expected if a catalyst for the oxidation of citrate such as acetoacetyl-coenzyme A has to be formed after the mixing of the components of the reaction mixture.

Chappell and Greville[23] have recently reported that the oxidation of isocitrate by rat-liver mitochondria is inhibited by the addition of lewisite or by the removal of phosphate in the presence of dinitrophenol (see also ref. 24), procedures known to inhibit the oxidation of α-ketoglutarate. They conclude that oxaloacetate formed by the further oxidation of the α-ketoglutarate is necessary for the oxidation of the mitochondrial TPNH. This suggestion was supported by their finding that, in the presence of lewisite, catalytic amounts of malate promoted the oxidation of isocitrate.

It is possible that the system in rat-liver mitochondria is different from that in rabbit-heart sarcosomes. The reaction conditions used in this study were also different from those used by Chappell and Greville. In any event, it seems unlikely that oxaloacetate is functioning directly to oxidize mitochondrial TPNH in my experiments for the following reasons: (i) acetate decreases the concentration of oxaloacetate, as made evident by the stimulation by acetate of the oxidation of succinate, which is inhibited by oxaloacetate, and by the inhibition by acetate of the oxidation of glutamate which requires oxaloacetate[25]; (ii) dinitrophenol which inhibits the oxidation of isocitrate causes increased concentrations of oxaloacetate[25]; (iii) glutamate which removes oxaloacetate by transamination also promotes the oxidation of isocitrate; (iv) malonate does not inhibit the α-ketoglutarate-promoted oxidation of isocitrate.

I wish to thank Prof. E. C. Slater for his suggestions and criticism and Miss H. M. Moerdijk for technical assistance.

This work was supported in part by research grant *RG*–6569 of the United States Public Health Service.

[1] Hülsmann, W. C., *Biochim. Biophys. Acta*, **45**, 623 (1960).
[2] Wakil, S. J., and Ganguly, J., *J. Amer. Chem. Soc.*, **81**, 2597 (1959).
[3] Wood, H. G., and Stjernholm, R., *Proc. U.S. Nat. Acad. Sci.*, **47**, 289 (1961).
[4] Montgomery, C. M., and Webb, J. L., *J. Biol. Chem.*, **221**, 347 (1956).
[5] Ziegler, D. M., and Linnane, A. W., *Biochim. Biophys. Acta*, **30**, 53 (1958).
[6] Kaplan, N. O., Swartz, M. N., Frech, M. E., and Ciotti, M. M., *Proc. U.S. Nat. Acad. Sci.*, **42**, 481 (1956).
[7] Purvis, J. L., *Biochim. Biophys. Acta*, **30**, 440 (1958).
[8] Klingenberg, M., and Slenczka, W., *Biochem. Z.*, **331**, 486 (1959).
[9] Langdon, R. G., *J. Biol. Chem.*, **226**, 615 (1957).
[10] Seubert, W., Freull, F., and Lynen, F., *Angew. Chemie*, **69**, 359 (1957).
[11] Wakil, S. J., Titchener, E. B., and Gibson, D. M., *Biochim. Biophys. Acta*, **34**, 227 (1959).
[12] Lehninger, A. L., and Greville, G. D., *Biochim. Biophys. Acta*, **12**, 188 (1953).
[13] Stern, J. R., Del Campillo, A., and Lehninger, A. L., *J. Amer. Chem. Soc.*, **77**, 1073 (1955).
[14] Wakil, S. J., *Biochim. Biophys. Acta*, **18**, 314 (1955).
[15] Lynen, F., *J. Cell. Comp. Physiol.*, **54**, supp. 33 (1959).
[16] Martin, D. B., Horning, M. G., and Vagelos, P. R., *J. Biol. Chem.*, **236**, 663 (1961).
[17] Stern, J. R., Coon, M. J., and Del Campillo, A., *Nature*, **171**, 28 (1953).
[18] Green, D. E., Goldman, D. S., Mii, S., and Beinert, H., *J. Biol. Chem.*, **202**, 137 (1953).
[19] Walker, P. G., *Biochem. J.*, **58**, 699 (1954).
[20] Hülsmann, W. C., *Over het mechanisme van de ademhalingsketen phosphorylering*, M.D. thesis (Klein Offset Drukkerij Poortpers, Amsterdam, 1958).
[21] Borst, P., and Slater, E. C., *Biochim. Biophys. Acta*, **41**, 170 (1960).
[22] Menon, G. K. K., and Stern, J. R., *J. Biol. Chem.*, **235**, 3393 (1960).
[23] Chappell, J. B., and Greville, G. D., *Nature*, **190**, 502 (1961).
[24] Borst, P., and Slater, E. C., *Biochim. Biophys. Acta*, **48**, 362 (1961).
[25] Slater, E. C., and Hülsmann, W. C., *Proc. U.S. Nat. Acad. Sci.*, **47**, 1109 (1961).
[26] Holton, F. A., Hülsmann, W. C., Myers, D. K., and Slater, E. C., *Biochem. J.*, **67**, 579 (1957).

REVERSAL OF TOXICITY OF 5-FLUOROURACIL AND 5-FLUORODEOXYURIDINE FOR *CANDIDA ALBICANS* BY PYRIDOXINE AND PYRIDOXAMINE

By Dr. M. L. LITTMAN and Dr. T. MIWATANI

Department of Microbiology, Mount Sinai Hospital, New York, and Osaka University Medical School, Osaka, Japan

DURING work on the metabolism of the pathogenic yeast, *Candida albicans*, we observed that the growth of this organism in a synthetic glucose mineral salt medium was markedly inhibited by the anti-cancer fluorinated uracil derivatives, 5-fluorouracil (5-FU, mol. wt. 130) and 5-fluorodeoxyuridine (5-FUDR, mol. wt. 246·2). (These compounds were kindly supplied by Hoffmann-La Roche, Inc., Nutley, New Jersey.) In following the inhibitory action of these compounds, we noted also that such effects could be promptly reversed by the addition of pyridoxine and pyridoxamine. In view of the high toxicity of the 5-fluoropyrimidines and the absence of clinically effective reversing compounds other than thymidine for 5-FUDR (ref. 1), application of this observation *in vivo* in human and animal cancer therapy seems to be indicated.

The basal synthetic culture medium, in which growth, growth-inhibition and inhibition-reversal occurred, contained the following ingredients: glucose, 10 gm.; ammonium sulphate, 2 gm.; potassium dihydrogen phosphate, 2 gm.; crystalline magnesium chloride, 0·2 gm.; crystalline calcium chloride, 0·02 gm.; crystalline ferrous chloride, 0·04 gm.; crystalline manganous chloride, 0·0015 gm.; crystalline sodium molybdate, 0·0015 gm.; and biotin, 1,000 mμgm., all dissolved in 900 ml. potassium permanganate-oxidized double distilled

water, after which the medium was adjusted to neutrality with 1 *N* sodium hydroxide. The basal medium was distributed in 9-ml. volumes to optically matched, chemically clean 'Lumetron' glass tubes which were covered with aluminium caps and sterilized by autoclaving at 118° C. (12 lb.) for 12 min. Glass filter-sterilized solutions of varying concentrations of 5-FU, 5-FUDR and vitamin B_6, each contained in a volume of 1 ml., were then added aseptically. After being seeded, tubes were incubated at 28° C. in a New Brunswick Gyrotory Shaker under constant rotation at 250 cycles per min. Daily turbidity measurements were made photometrically with a 401*A* 'Lumetron' colorimeter at 650 mμ. The standard inoculum consisted of vitamin-depleted yeast cells prepared by subculturing the organism twice on vitamin-free basal synthetic culture medium agar slopes, with an intervening 7-day incubation period at 37° C. By the time of the second sub-culture, growth of the organism had become sparse. Vitamin-free washed agar incorporated in the formula was prepared by a previously described method[2]. Vitamin-depleted yeast cell suspensions were collected and washed three times in sterile, double-distilled water, adjusted to 50 per cent light transmission at 650 mμ and then inoculated at a standard dose of 0·15 ml. suspension per tube.

Inhibitory effect of 5-FU and 5-FUDR. Ascending concentrations of 5-fluoropyrimidines caused a progressive restriction of growth of *C. albicans* to the point of complete inhibition in the case of 5-FU (Fig. 1) and 60–70 per cent inhibition by 5-FUDR. This inhibitory effect was observable within the first day of incubation and persisted for the entire 5-day observation period. Some escape from the inhibitory effect of 60–90 μgm. 5-FU/ml. appeared by the fourth day of incubation (Fig. 1). However, no escape from inhibition occurred from the effects of 5-FUDR over the same observation period. 50 per cent minimal inhibiting dose (ID_{50}) of 5-FU varied from 31 to 37 μgm./ml. (0·24–0·28 μmole/ml.) from the second to fifth day of incubation, and averaged 34·3 μgm./ml. (0·26 μmole/ml.). The inhibitory action of 5-FUDR on *C. albicans* was approximately ten times that of 5-FU, although, unlike the latter compound, it never produced complete restriction of growth at any concentration. The ID_{50} of 5-FUDR

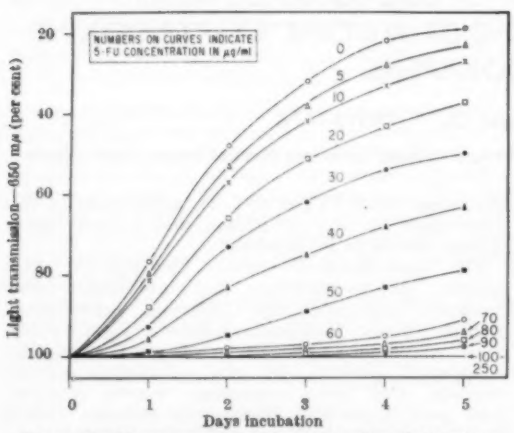


Fig. 1. Growth response of *C. albicans* in basal synthetic culture medium to increasing concentrations of 5-FU at 28° C. in incubator shaker at 250 cycles/min. Each point represents an average of triplicate values, arithmetic plot

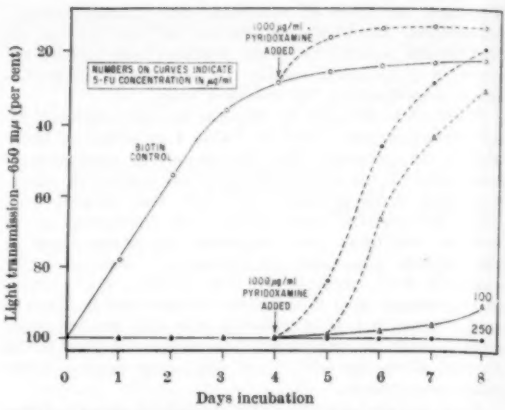


Fig. 2. Reversing effect of pyridoxamine (1,000 μgm./ml.) on growth-inhibition of cells of *C. albicans* previously exposed for four days to high concentrations of 5-FU in basal synthetic culture medium at 28° C. in incubator shaker at 250 cycles/min. Each point represents an average of triplicate values, arithmetic plot

varied from 3·9 to 1·86 μgm./ml. (0·03–0·014 μmole/ml.) from the second to fifth day of incubation, and averaged 2·9 μgm./ml. (0·022 μmole/ml.).

Reversing effect of pyridoxine and pyridoxamine. Stimulated by the newly observed fact that five representative strains of *C. albicans* had all shown a partial deficiency for pyridoxamine at an optimal concentration of 1,000 μgm./ml. (ref. 3), which is beyond the usual concentration for vitamins, we considered the possibility that vitamin B_6 could be involved in cell metabolism of *C. albicans* other than as a coenzyme in amino-acid metabolism. We therefore applied these vitamins to viable but growth-inhibited yeast suspensions containing ascending concentrations of both 5-FU and 5-FUDR. We then observed that the growth-inhibiting effects of the two 5-fluoropyrimidines were reversed by pyridoxine as well as pyridoxamine. To our knowledge, this phenomenon has not been reported previously. As shown in Fig. 2, the addition of 1,000 μgm. pyridoxamine/ml. (4·1 μmoles/ml.) to incubating yeast cells on the fourth day of their continuous exposure to varying concentrations of 5-FU brought about a prompt reversal of growth inhibition. The more pronounced reversals occurred at the higher concentrations of 5-FU (50–250 μgm./ml.) than lower concentrations. Reversal of 5-FU inhibition by pyridoxamine was complete in that final turbidities in pyridoxamine-reversed tubes were approximately equal to, or exceeded, those of biotin control tubes not containing 5-FU (Fig. 2).

Extracellular pigment formation. A water-soluble, extracellular, pigmented metabolic product of *C. albicans*, which is ordinarily not a pigmented yeast, appeared regularly in all seeded tubes of basal synthetic culture medium containing 0·25 μmole 5-FU/ml. or higher, whenever the concentration of pyridoxamine exceeded 5 μmoles/ml. The coloured product diffused into the supernatant fluid after 24 hr. of incubation and progressively increased in concentration with time. Pigment was not produced when pyridoxine was substituted for pyridoxamine nor when 5-FU and pyridoxamine were shaken together in distilled water. Substitution of 5-FUDR for 5-FU yielded a similar pigment with pyridoxamine, but not with pyridoxine. This also occurred after the first day of incubation in tubes containing

10 mμmoles 5-FUDR/ml. or higher with at least 5,000 mμmoles pyridoxamine/ml.

Partial purification of the pigment produced by *C. albicans* from 5-FU and pyridoxamine was accomplished by de-ionization of filtered supernatant fluid with '*IRC*-50' cationic exchanger, adsorption at *p*H 3 with 'Darco *G*60' activated charcoal, followed by several washings with distilled water, after which the pigment was eluted from the charcoal with alkaline 50 per cent ethanol, to yield a clear yellow solution. The pigmented product behaved as an indicator in this solution:

*p*H	Colour
< 1	No colour
2	Light reddish violet
2·8–3·5	Red
5·5–6·5	Yellowish pink
6·5–11	Yellowish brown
> 12	Yellow

The yellow solution was adjusted with hydrochloric acid to a red colour at *p*H 3. In ascending paper chromatograms in *n*-butanol/methanol/benzene/water = 2 : 1 : 1 : 1 (upper layer) for 15 hr. at 22° C., the red-coloured product, not fluorescent in ultra-violet light, migrated to R_F 0·43, interspersed, but clearly separable, from five other fluorescent unpigmented spots. When tested against *C. albicans*, the pigmented, metabolic product was neither growth-inhibitory nor growth-stimulatory in seeded basal synthetic culture medium agar. Further purification and characterization of this pigment is proceeding.

Order of introduction of metabolite versus antimetabolite in test system. Further experiments were conducted to determine whether the order of addition of the metabolite and antimetabolite was crucial for the reversal action of the metabolite. Pyridoxine and pyridoxamine were introduced simultaneously, first with 5-FU and then with 5-FUDR, into basal synthetic culture medium, followed immediately by addition of the standard inoculum of vitamin-depleted yeast cells. Under these new conditions, pyridoxamine (Fig. 3) and pyridoxine were again capable of reversing the toxicity of 5-FU for *C. albicans*. Substitution of 5-FUDR produced similar results except that pyridoxamine was more effective in reversing 5-FUDR inhibition than was pyridoxine. The order of addition of vitamin B_6 and the 5-fluoropyrimidines is therefore not crucial. Vitamin B_6 reversed the toxicity of 5-FU for cells in prior contact with 5-FU for 4 days (Fig. 2), as well as for cells previously unexposed to 5-FU (Fig. 3).

Reversal of 5-FU inhibition by vitamin B_6 using 5-FU exposed and unexposed cells. Inocula in the preceding experiments had consisted of vitamin-depleted yeast cells previously unexposed to 5-fluoropyrimidines or to vitamin B_6. We now wished to determine whether yeast cells continually exposed to 60 μgm. 5-FU/ml. for a 7-day period at 28° C. would respond to the reversing action of vitamin B_6 in the same manner as unexposed cells. Accordingly, yeast cells that had been incubated 7 days in the shaker in the presence and absence of 5-FU were collected, washed three times with sterile, double-distilled water, re-suspended, and adjusted to 50 per cent light transmission. These 7-day-old cells were re-inoculated into fresh tubes of basal synthetic culture medium containing 5-FU, with and without 1,000 μgm. pyridoxamine/ml. As seen in Figs. 4 and 5, a 7-day prior period of incubation, whether in the presence of 60 μgm. 5-FU/ml. or not, was capable of

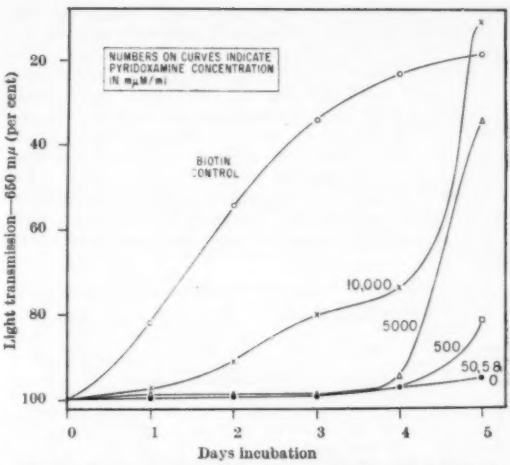


Fig. 3. Growth response of *C. albicans* and the reversing effect of five levels of pyridoxamine on growth inhibition by 5-FU at 28° C. in incubator shaker in basal synthetic culture medium at 250 cycles/min. All tubes contain 1,000 mμmoles 5-FU/ml. except biotin control. Metabolite and antimetabolite introduced together. Each point represents an average of triplicate values, arithmetic plot

exhausting the reproductive ability of *C. albicans* and prolonging the lag phase of growth to 3 days (biotin control curves). However, when pyridoxamine was added with biotin, the prolonged lag phase was eliminated regardless of whether the cells had been exposed to 5-FU or not (biotin + pyridoxamine curve). This action of pyridoxamine points to it as having an important role in the cellular reproduction of *C. albicans*.

5-FU exposed yeast cells were completely inhibited by second contact with 5-FU over the range 250–2,000 mμmoles/ml. within the 5-day incubation period (Fig. 5). However, appreciable escape from inhibition by 5-FU began to occur by the sixth and seventh day, signalling what we interpret as the emergence of 5-FU adapted or resistant cells. In the case of unexposed cells, there was also complete inhibition by 5-FU over the same range of concentration, but no escape occurred over a 7-day incubation period (Fig. 4). Thus, prior conditioning to 5-FU appears to favour the emergence of 5-FU adapted or resistant cells. The future usefulness of 5-FU resistant *C. albicans* cells as an indicator system for screening useful compounds for 5-FU-resistant cancer lines, remains to be determined. In comparing the speed of reversal by pyridoxamine of growth inhibition of 5-FU exposed yeast cells and unexposed cells, one notes more rapid reversal of growth inhibition of exposed cells than that of unexposed cells (Figs. 4 and 5). This may be attributed to : (1) the adaptation or increased resistance to 5-FU of the yeast cell exposed twice to 5-FU; (2) to the accumulation of unused growth-stimulatory intermediate metabolites blocked by the prior contact with 5-FU.

Type of antagonism between vitamin B_6 and 5-fluoropyrimidines. In order to determine whether the antagonism between vitamin B_6 and the 5-fluoropyrimidines was competitive or non-competitive, a determination was made of the amounts of 5-FU and 5-FUDR which were required to cause half-maximal inhibition of growth in the presence of ascending ten-fold concentrations of pyridoxine and pyridoxamine (Tables 1 and 2). Since the

order of addition of metabolite and antimetabolite was not crucial, both vitamin B_6 and 5-fluoropyrimidines were introduced together with the inoculum. As seen in Tables 1 and 2, inhibition indices for both 5-FU and 5-FUDR for *C. albicans* were small, indicating very high antimetabolite activity of the 5-fluoropyrimidines in the *C. albicans* test system. However, the inhibition indices were not constant for either pyridoxine or pyridoxamine versus 5-FU or 5-FUDR and the antagonism was non-competitive. This would indicate that vitamin B_6 competes with the 5-fluoropyrimidines at more than one site on the cell.

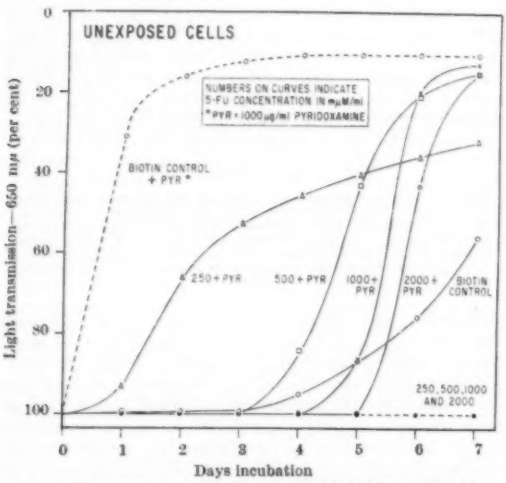


Fig. 4. The reversing action of pyridoxamine (1,000 μgm./ml.) on growth inhibitory effect of 5-FU on previously unexposed cells of *C. albicans* at four levels of 5-FU at 28° C. in basal synthetic culture medium in incubator shaker at 250 cyles/min. Metabolite and antimetabolite introduced together. Biotin control tubes, with and without pyridoxamine, do not contain 5-FU. Note complete inhibition without escape, by 250, 500, 1,000 and 2,000 mμmoles 5-FU/ml., in absence of pyridoxamine. Each point represents an average of triplicate values, arithmetic plot

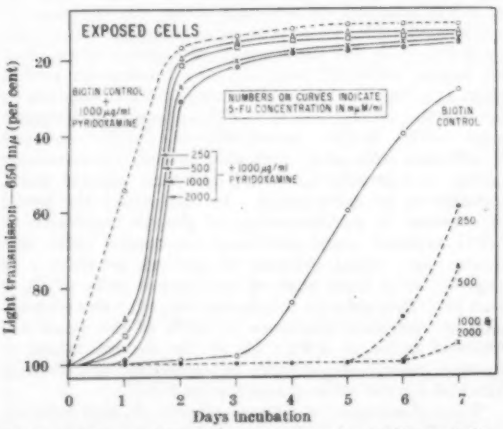


Fig. 5. The reversing action of pyridoxamine (1,000 μgm./ml.) on growth inhibition of 5-FU on cells of *C. albicans* previously in continuous contact for seven days with an 80 per cent inhibitory dose of 5-FU (0·30 μmole/ml.). Test run at four levels of 5-FU at 28° C. in basal synthetic culture medium in incubator shaker at 250 cycles/min. Metabolite and antimetabolite introduced together. Biotin control tubes, with and without pyridoxamine, do not contain 5-FU. Note complete inhibition with escape in tubes containing 250, 500, 1,000 and 2,000 mμmoles 5-FU/ml., in absence of pyridoxamine. Each point represents an average of triplicate values, arithmetic plot

Table 1. Amounts of 5-FU required to produce Half-maximal Inhibition of Growth of *Candida albicans* in the Presence of Varying Amounts of Pyridoxamine and Pyridoxine

(Four days incubation at 28° C. in New Brunswick Gyrotory Shaker, 250 cycles/min.)

Metabolite (μmoles/ml.)	5-FU μmoles/ml. (ID_{50})	Inhibition index *
Pyridoxamine		
0	0·27	—
0·005	0·27	—
0·05	0·28	0·28
0·5	0·31	0·08
5·0	0·59	0·06
10·0	0·63	0·04
Pyridoxine		
0	0·27	—
0·005	0·27	—
0·05	0·31	0·8
0·5	0·32	0·1
5·0	0·72	0·09
10·0	0·77	0·05

* Inhibition index = (ID_{50} of 5-FU in presence of metabolite) minus (ID_{50} in absence of metabolite) divided by (concentration of metabolite).

Table 2. Amounts of 5-FUDR required to produce Half-maximal Inhibition of Growth of *Candida albicans* in the Presence of Varying Amounts of Pyridoxamine and Pyridoxine

(Four days incubation at 28° C. in New Brunswick Gyrotory Shaker, 250 cycles/min.)

Metabolite (μmoles/ml.)	5-FUDR (μmoles/ml.) (ID_{50})	Inhibition index *
Pyridoxamine		
0	0·021	—
0·05	0·023	0·04
0·5	0·019	0·004
5·0	3·00	0·6
Pyridoxine		
0	0·021	—
0·05	0·021	—
0·5	0·021	—
5·0	0·029	0·0016

* Inhibition index = (ID_{50} of 5-FUDR in presence of metabolite) minus (ID_{50} in absence of metabolite) divided by (concentration of metabolite).

The 5-fluoropyrimidines inhibit mammalian tumours by interfering with synthesis of ribonucleic (RNA) and deoxyribonucleic (DNA) acids[4–6]. They inhibit incorporation of formate, uracil-4-carboxylic acid and pyrimidines into DNA thymine of liver, spleen and the Ehrlich tumour *in vivo*. They also block the incorporation of uracil and uracil-4-carboxylic acid into RNA uracil. The most important site of action is in the inhibition of methylation of uridylic acid to form thymidylic acid[5]. Analogous effects of thymine and uracil metabolism have been shown to occur in microbiological systems, such as *Escherichia coli*[7], *Staphylococcus aureus*[8], and others. It is reasonable, therefore, to postulate that at least one of the inhibitory effects of 5-FU and 5-FUDR on *C. albicans* is due to interference with nucleoprotein synthesis in this organism. Further work, however, will be required to confirm this hypothesis.

In view of the fact that the growth-restricting action of 5-FU and 5-FUDR on *C. albicans* was reversed by vitamin B_6, and that growth of the organism continued to be restricted by increasing concentrations of 5-fluoropyrimidines despite the presence of optimal concentrations of vitamin B_6, we can assume that the 5-fluoropyrimidines interfere in some manner with uptake or utilization of vitamin B_6 by the yeast cell, unless the vitamin was itself involved in nucleoprotein synthesis. We are not aware of any reports that vitamin B_6 is thus involved. We did observe that when exhausted cells of *C. albicans*, both exposed and unexposed to the prior inhibitory effects of 5-FU, were given pyridoxamine, the unduly prolonged lag phase of the organism promptly disappeared (Figs. 4 and 5), indicating a salutary effect on cellular reproduction.

The presence of pyridoxal and pyridoxal phosphate in the vicinity of isolated Ehrlich ascites tumour cells intensifies their uptake of amino-acids, both under aerobic[9] and anaerobic[10] conditions. These tumour cells quickly take up pyridoxal to an extent calculated to be 1·3–1·4 times that of the extracellular level of the vitamin[11]. This is understandable, since Ehrlich ascites tumour cells have been reported to have a greater capacity to take up amino-acids from the blood and concentrate them, than have normal cells[12]. These findings are also compatible with the known primary functions of vitamin B_6 as a coenzyme in amino-acid decarboxylation, transamination and catalytic breakdown of tryptophan. Our observations of the reversing effect by pyridoxine and pyridoxamine on 5-FU and 5-FUDR growth inhibition of *C. albicans* directs attention to the possibility of a new function of vitamin B_6 in nucleic acid metabolism, as well as a possible new mode of action of the 5-fluoropyrimidines. The antagonism between vitamin B_6 and 5-fluoropyrimidines also raises the question as to whether 5-fluoropyrimidine-resistant cancer cells possess a 'built-in' resistance mechanism by virtue of possessing greater levels of B_6 or by their synthesizing greater quantities of the vitamin than do susceptible cancer cells. The effect of vitamin B_6 on 5-fluoro-pyrimidine-intoxicated animals, with and without transplanted tumours, in order to determine the ability of vitamin B_6 to reduce parenteral toxicity of the 5-fluoropyrimidines, is the next area of exploration under consideration. Investigations along some of these lines are under way.

This investigation was supported by a research grant, *G*-7072, of the National Science Foundation, Washington, D.C.

[1] Miller, E., Sullivan, R., Young, C., and Burchenal, J. H., *Proc. Amer. Assoc. Cancer Res.*, **3**, 251 (1961).
[2] Littman, M. L., *Trans. N.Y. Acad. Sci.*, Ser. II, **20**, 623 (1958).
[3] Littman, M. L., and Miwatani, T., Second Conference on Experimental Clinical Cancer Chemotherapy, Washington, D.C., November 2–3, 1961.
[4] Eidinoff, M. L., Knoll, J. E., and Klein, D., *Arch. Biochem. Biophys.*, **71**, 274 (1957).
[5] Danneberg, P. B., Montag, B. J., and Heidelberger, C., *Cancer Res.*, **18**, 329 (1958).
[6] Chaudhuri, N. K., Montag, B. J., and Heidelberger, C., *Cancer Res.*, **18**, 318 (1958).
[7] Cohen, S. S., Flaks, J. G., Barner, H. D., Loeb, M. R., and Lichenstein, J., *Proc. U.S. Nat. Acad. Sci.*, **44**, 1004 (1958).
[8] Rogers H., J., and Perkins, H. R., *Biochem. J.*, **74**, 6*P* (1960).
[9] Christensen, H. N., Riggs, T. R., and Coyne, B. A., *J. Biol. Chem.*, **209**, 413 (1954).
[10] Oxender, D. L., and Christensen, H. N., *J. Biol. Chem.*, **234**, 2321 (1959).
[11] Pal, P. R., and Christensen, H. N., *J. Biol. Chem.*, **236**, 894 (1961).
[12] Christensen, H. N., and Henderson, M. E., *Cancer Res.*, **12**, 229 (1952).

'GLYCEROL EFFECT' AND THE MECHANISM LINKING EXCITATION OF THE PLASMA MEMBRANE WITH CONTRACTION

By Prof. MASAHIRO FUJINO, TOSHIO YAMAGUCHI and KEISHI SUZUKI

Department of Physiology, Sapporo Medical College, Sapporo, Japan

FOR various reasons, it seemed of interest to consider the use of glycerol and/or other non-electrolyte in investigating the mechanism of a 'membrane'. Thus: (1) in the biochemical and biophysical fields[1,2], glycerol has been used for disintegrating the cell membrane, suggesting the possibility that it might have an effect on other membrane systems of the muscle cell, for example, sarcoplasmic reticulum[3]; (2) glycerol is a constituent member of the lipid part of lipoprotein, which seems to form the framework of the membrane system of the cell[4]. We have therefore investigated the relationship between glycerol and the activity of muscle, in an attempt to elucidate the mechanism of the excitation-contraction link of the muscle cell, using the 'glycerol effect' for this purpose.

If a single muscle fibre from the frog's skeletal muscle (m. tibiofibularis and m. semitendinosus of *Rana nigromaculata* or *Rana japonica*) is immersed in a Ringer's fluid which is hypertonic due to the addition of a high concentration of glycerol, say, about 420 m*M*, the mechanical response in twitch declines and then disappears within about 1 min. (Fig. 1*A*2). Nevertheless, such fibres are electrically excitable, and the action and resting potentials are almost unchanged (Fig. 1*B*2), though the rate of rise and fall of the action potential is likely to be slightly reduced. Since the diminution of the mechanical response due to the hypertonic condition and the differential action on the mechanical response and action potential of a muscle fibre have been already reported[5,6], this effect as such is not new. The disappearance of the mechanical response is considered to be due not to the chemical effect of glycerol but to the hypertonicity of the Ringer's fluid used; this view is supported by the fact that the disappearance of the response is accompanied by a considerable loss of weight of muscle (Fig. 2).

About 6–10 min. after immersion in Ringer's fluid containing glycerol, however, a new observation, which we have called the 'glycerol effect', appears. As shown in Fig. 1*A*, in spite of being left in the hypertonic condition, mechanical response in the form of twitch re-appears, the twitch tension rises gradually and reaches 50–70 per cent (maximum value) of the normal within 40–60 min. after re-appearance. It is very interesting that the restoration takes place without any further changes in either action or resting potential (Fig. 1*B*) and even in the weight of muscle (Fig. 2).

These results indicate directly that the disappearance of the ability to develop twitch tension under hypertonic conditions occurs not by alteration of the contractile system due to dehydration, but by paralysis of function of a strategic system linking the excitation of the plasma membrane with the contraction.

A similar phenomenon, namely, the disappearance of the mechanical response followed by its re-appearance is also recognizable in the case of complete tetanus, which is evoked at appropriate intervals to test the contractibility.

Experiments with the isotonic lever on whole muscle of the frog sartorius give the following results:

(1) The time of re-appearance of the twitch and the gradation of the twitches are intimately related to the duration for which the muscle is immersed in Ringer's fluid containing glycerol, and are not affected by the intervals (1–9 min.) at which a stimulation to evoke twitch is given. (2) This phenomenon is recognizable over the wide range of glycerol concentrations (120–840 mM), though it becomes obscure at both extremes of concentration—at the higher concentrations, due to the reduced contraction of the re-appeared twitch; at the lower one, due to the small diminution of the mechanical response. (3) Under various hypertonic conditions, produced by adding sodium chloride (150–250 mM) or several non-electrolytes (mannitol, 50–400 mM; monoacetin $CH_2OH{\cdot}CHOH{\cdot}CH_2OCOCH_3$, 50–300 m$M$; glucose, 200–400 m$M$; sucrose, 200–300 m$M$, and urea, 300 m$M$) to normal Ringer's fluid, only diminution or abolition of twitch occurs; hence the re-appearance of twitch after diminution is highly specific to glycerol, and the term the 'glycerol effect' is quite justified. (4) The 'glycerol effect' appears also in the case of sartorius muscle (13 mM potassium chloride) depolarized with potassium, when stimulated in either a longitudinal or a transverse a.c. (50 c./s.) electric field (8–10 V./cm., 40–94 msec.). Another series of experiments showed that the 'glycerol effect' occurs in whole muscle of the frog rectus abdominis, whereas it does not in the frog heart ventricle. We have found that the 'glycerol effect' is recognizable in Ringer's fluid without calcium or potassium or both.

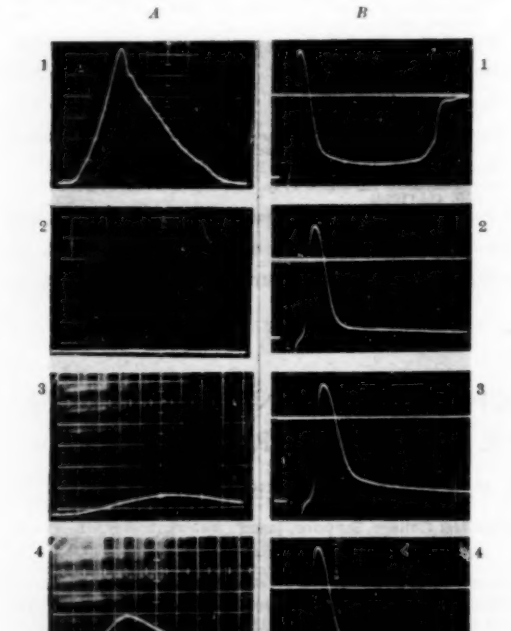


Fig. 1. Mechanical and electrical responses of single muscle fibres of the frog semitendinosus at different times after replacing the Ringer's fluid by the one containing 420 mM glycerol
The series of records A shows twitch tensions obtained from a single muscle fibre: $A1$, in normal Ringer's fluid; $A2$, $A3$, $A4$ and $A5$, after soaking in glycerol Ringer's fluid for 1 min., 24 min., 44 min. and 60 min., respectively. The series of records B shows action potentials obtained from single muscle fibres of a bundle: $B1$, in normal Ringer's fluid (dislodging the microelectrode caused by the movement of the fibre resulted in the displacement of the tracing to 0 mV. at the right-hand side); $B2$, $B3$, $B4$ and $B5$ after soaking in glycerol Ringer's fluid for 3 min., 26 min., 45 min. and 60 min., respectively
The fibres were stimulated by means of a pair of external electrodes with a square pulse generated from an electronic stimulator at room temperature (17° C.). The tension recorder was a mechano-electronic transducer *RCA* 5734, and glass capillary micro-electrodes filled with 3 M potassium chloride (10–30 megohms) were employed to record action potentials. The Ringer's fluid contained 122 mM sodium chloride, 2 mM potassium biphosphate, 1·3 mM calcium chloride and was buffered to pH 7·0 with sodium bicarbonate

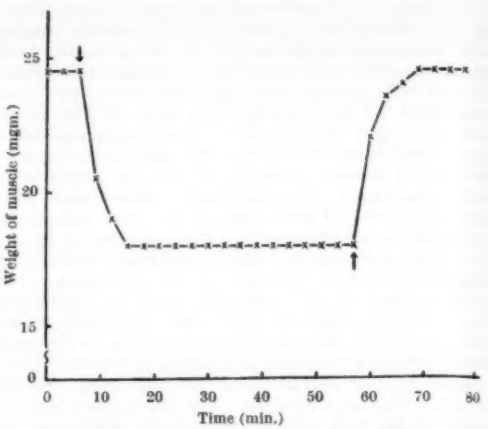


Fig. 2. Osmotic effect of hypertonic Ringer's fluid containing 420 mM glycerol on whole muscle of frog sartorius
At ↓, the bathing fluid was exchanged with the glycerol Ringer's fluid, and at ↑, the normal Ringer's fluid was restored. The muscle was weighed on a torsion balance after it was blotted carefully with pieces of filter paper

From these results it is clear that the re-appearance of the mechanical activity takes place as the result of the effect of glycerol on a significant process (or processes) which links the membrane excitation with the contraction and is paralysed under the hypertonic condition. Since glycerol is not considered to diffuse into a muscle fibre, at least within the 10–20 min. in which the 'glycerol effect' begins to appear (no further detectable change in muscle weight occurs at this time (Fig. 2); Bozler, E., personal communication), it is believed that this process exists at the plasma membrane or at its vicinity. It follows also that the contraction both in the transverse a.c. field and in the longitudinal field would not be due to any internal currents flowing within a muscle fibre, but to activation of a process at the membrane or in its vicinity[7,8].

How this 'glycerol effect' occurs is at present difficult to understand. The following explanation is very speculative: we have already pointed out the possibility of the existence of a synapse-like process in the sequence of processes linking excitation with contraction[9]. On the other hand, it is probable, according to Huxley[10], that the Z-line or the endoplasmic reticulum is the structure responsible for the electrical spread of excitation in question. It

follows then that there would be two synapse-like sites in this linking mechanism: one is in the vicinity of the plasma membrane or between the plasma membrane and the endoplasmic reticulum, and the other between the reticulum and the contractile system. The 'glycerol effect' would be chemically (for this effect is highly specific to glycerol) or osmotically related to the former.

Details of this work are to be published elsewhere, with further experimental evidence.

We have found, in addition, a conspicuous effect. If, in these experiments, the normal Ringer's fluid is restored, the development of tension in the fibre by electrical stimulation is reduced and then abolished within a few minutes, in spite of the production of the vigorous action potential. We have no explanation at present of this effect.

We thank Prof. T. Nagai for his constant advice and Prof. E. Miyazaki for his encouragement. We are also grateful to Dr. T. Matsushima, Miss K. Abe and Mr. T. Yamamoto for assistance.

[1] Szent-Györgyi, A., *Chemistry of Muscular Contraction*, second edit. (Acad. Press, New York, 1951).
[2] Haurowitz, F., *Chemistry and Biology of Proteins* (Acad. Press, New York, 1950).
[3] Porter, K. R., and Palade, G. E., *J. Biophys. Biochem. Cytol.*, **3**, 269 (1957).
[4] Danielli, J. F., *Surface Phenomena in Chemistry and Biology*, edit. by Danielli, J. F., Pankhurst, K. G. A., and Riddiford, A. C., 246 (Pergamon Press, London, 1958).
[5] Hodgkin, A. L., and Horowicz, P., *J. Physiol.*, **136**, 17*P* (1957).
[6] Howarth, J. V., *J. Physiol.*, **144**, 167 (1958).
[7] Csapo, A., and Suzuki, T., *J. Gen. Physiol.*, **41**, 1083 (1958).
[8] Sten-Knudsen, O., *J. Physiol.*, **151**, 363 (1960).
[9] Fujino, M., Matsushima, T., Muroya, T., Yabu, H., Yamaguchi, S., and Takahashi, M., *Nature*, **186**, 318 (1960).
[10] Huxley, A. F., *Ann. N.Y. Acad. Sci.*, **81**, 446 (1959).

MIXED SALIVA FROM SHEEP

By W. R. McMANUS

School of Wool Technology, University of New South Wales, Sydney

IT is well known that the parotid glands of adult ruminants produce saliva continuously and their output volumetrically dominates the relatively smaller contribution made by the other salivary glands. Most reports agree that the submaxillary and sublingual glands chiefly function during the consumption of feed. The buccal glands have received little investigation. The saliva arising from the four types of glands is chemically different. There are few reports in the literature on the properties and flow-rates of mixed ovine saliva, especially on the quantity of endogenous nitrogen re-cycled to the rumen.

In many nutritional investigations on sheep there is need for information on the volume of saliva passing to the reticulo-rumen. Denton[1], using parotid fistulated sheep, has noted values for the flow of parotid saliva of animals consuming roughage and succulent rations. The observations of Bath *et al.*[2], conducted with one œsophageal fistulated sheep, have indicated the volume of mixed saliva put out while this animal consumed lucerne chaff—but the interval of time was not recorded.

The two experiments reported here were made during the course of a series of basic investigations on sheep fitted with chronic œsophageal fistulæ[3]. The fistulæ differed in area (2·0–8·0 cm.²) and were all well healed.

Experiment 1 is concerned with minimal estimates of the volume of mixed saliva passing to the reticulo-rumen of sheep while consuming two types of roughage rations. Experiment 2 compares the composition and flow-rates of mixed saliva of anæsthetized sheep and conscious sheep while not consuming feed.

Table 1 shows the flow-rates of mixed saliva achieved on nine occasions in experiment 1 by five œsophageal fistulated sheep when consuming either a 55 per cent wheat grain, 25 per cent wheaten chaff and 20 per cent lucerne chaff ration (diet 1), or good quality lucerne chaff (diet 2) of known moisture contents. Information on duration of sampling, amount of ration consumed and the percentage recovery, via fistula, of extruded feed are also given.

Fistula extrusa was collected in polythene bags fitted around the animals' neck. Saliva flow-rates were estimated in the following manner. The 'free' saliva fluid was carefully separated from the particulate matter of the extrusa by compression and the fluid volume measured. The 'wet' particulate matter was weighed, oven-dried at 100° C. overnight and the moisture loss noted. After correction for the initial feed moisture content, the fluid volume difference between the 'wet' and 'dry' feed extrusa was added to the 'free' (saliva) fluid volume.

It is considered that this summated value represents the volume of saliva extruded from the fistulæ over the sampling period.

All attempts to occlude the œsophagus below the fistula, so as to ensure complete recovery of swallowed matter via fistulæ, resulted in distress to the animal. In experiment 1 an unknown amount of saliva by-passed the fistula and with the food entered the reticulo-rumen. Therefore these results do not represent estimates of the maximum volumes of saliva secreted by these sheep while consuming feed. However, they do reflect rates of mixed saliva secretion that can be achieved, and exceeded, by sheep while eating these roughage rations (mean 8·5 ml./min.; range, 3·8–12·0 ml./min.). In contrast, the mean saliva flow-rate for conscious œsophageal fistulated sheep (not eating, experiment 2) was only half as great (4·3 ml./min.; range, 0·52–17·0 ml./min.).

For experiment 2, six œsophageal fistulated and three non-fistulated animals were used. Twenty-one observations were made on the diversion and collection of mixed saliva from either conscious fistulated sheep (without concomitant consumption of feed), or orally from anæsthetized non-fistulated sheep ('Kemithal', lateral recumbency). For conscious sheep the collection periods ranged from 6 to 335 min. (mean 71·7 min.), and for the anæsthetized sheep from 268 to 370 min. (mean 300·2 min.).

The experimental conditions for the fistulated sheep varied widely between occasions. Some animals were fasted for 12 hr. before sampling, others were fed immediately before sampling. In some cases food was within the animals' sight—but in no case was the animal consuming its ration when saliva samples were collected. Some animals were more

Table 1. Rate of Extrusion of Mixed Saliva from Œsophageally Fistulated Sheep consuming Roughage Diets

Sheep and sampling date	Remarks	Duration of sampling (min.)	Weight of feed consumed (gm. dry matter)	Percentage recovery of extruded feed (dry matter basis)	Volume of saliva collected (ml.)	Rate of extrusion of saliva (ml./min.)
	Diet 1					
1 Oct. 14, 1955	Animal hungry and in good condition. Ate consistently	40	67·9	75	443	11·1
1 Oct. 17, 1955	Animal hungry and in good condition. Ate consistently	20	42·0	58	76	3·8
1 Oct. 19, 1955	Animal distressed due to fistula leakage. Hungry, losing weight. Ate consistently	38	58·3	95	291	7·7
1 Oct. 28, 1955	Animal distressed due to fistula leakage. Hungry, losing weight. Ate consistently	29	43·2	97	186	6·4
2 April 29, 1959	Animal hungry, ate consistently	25	178·3	71	163	6·5
3 April 29, 1959	Animal hungry, ate consistently	20	165·5	63	240	12·0
	Diet 2					
4 May 2, 1959	Animal hungry, ate consistently	50	38·6	53	515	10·3
5 May 1, 1959	Animal hungry, ate consistently	35	55·8	95	398	11·4
2 May 13, 1959	Animal hungry, ate consistently	70	141·4	39	526	7·5

Note: On no occasion reported here were fistulæ blocked either by 'packing' of feed, or by the polythene collection bags pressing against them.

docile than others. Before sampling, the mouth and the œsophagus were washed out with distilled water. At sampling, most, if not all, of the saliva passed via fistula from the conscious sheep and was collected. The fluid in these cases was almost invariably ejected as a cohesive volume rather than as a steady trickle. On the other hand, the experimental conditions of collection of saliva as it dripped from the gagged mouth of anæsthetized animals were almost constant. It is reasonable to conclude that collection of saliva from these sheep was complete. The mean concentration of sodium and potassium in the mixed saliva of one conscious, fistulated sheep (seven half-hourly sub-samples) were 138·5 m.equiv./l. and 35·7 m.equiv./l. respectively. The electrolyte composition of saliva from anæsthetized sheep was not examined.

For all values reported, other than ash and *p*H, there is a wider variation for conscious than for anæsthetized animals. Table 2 shows the mean values and their range. These findings agree substantially with other estimates appearing in the literature.

The large differences in rate of flow, total nitrogen and solids content between anæsthetized and conscious sheep are presumably due to the greater barrage of saliva eliciting stimuli received by the conscious sheep. Since the ash contents were similar, it is suggested that most of the difference in total solids content obtained for these two treatments is due to a difference in organic matter content—presumably mucoproteins. This is supported by the markedly higher (mean difference 13·2 mgm. per cent) nitrogen content of saliva from anæsthetized than from conscious animals.

A significant negative relationship obtained between flow-rate of mixed saliva and total nitrogen content for each of two anæsthetized sheep ($r = -0{\cdot}72$, $P < 0{\cdot}001$, 26 degrees of freedom, $r = -0{\cdot}67$, $P < 0{\cdot}001$, 20 degrees of freedom). There were insufficient observations per animal to permit any conclusions about the existence of a similar relationship for saliva from conscious animals.

These results relate to the diversion of mixed saliva from anæsthetized sheep for time periods of up to 6 hr. after consumption of a diet containing 11 per cent crude protein. They support Phillipson and Mangan's[4] suggestion that the nitrogen content of saliva could be diluted by increasing rates of fluid secretion.

On the other hand, Somers[5] found a positive relationship between rate of secretion and nitrogen content of mixed saliva ($r = 0{\cdot}9212$, $P < 0{\cdot}001$). His values are independent of time, mine depend on time. Therefore the two analyses are not comparable. In a later paper Somers[6], when examining a time-dependent relationship between total saliva nitrogen and parotid saliva volume secreted over a 24-hr. period for conscious sheep following feeding, showed an apparent positive relationship between these values. Diets of different nitrogen contents were used by Somers and by me. This, together with the different saliva fluids examined, could explain the two sets of findings.

Table 2. Showing Comparison between Certain Saliva Properties for Anæsthetized and Conscious Sheep
(Mean values)

Condition of animal	No. of sheep used	Flow-rate (ml./min.)	Nitrogen (mgm./100 ml.)	Solids (mgm./100 ml.)	Ash (mgm./100 ml.)	*p*H
Anæsthetized, non-fistulated	3					
Mean		2·52	20·73	1,253·9	662·6	8·52
Range		(0·30–8·30)	(18·01–28·12)	(560–1,518)	(240–1,164)	(8·24–8·65)
No. of values		(7	(54)	(33)	(33)	(43)
Conscious, fistulated	6					
Mean		4·31	7·52	969·8	609·8	8·24
Range		(0·52–17·0)	(1·75–14·64)	(146·0–1,265·0)	(64·0–840·0)	(8·06–8·47)
No. of values		(14)	(15)	(11)	(11)	(3)
Mean—all values		3·71	17·86	1,208·0	649·6	8·50

Table 3. Estimated Quantities of Mixed Saliva and of Contained Nitrogen entering the Ovine Rumen

State of animal	Mixed saliva output				Saliva nitrogen entering rumen			
	Flow-rate ml./hr.	Volume l./day	No. of observations	No. of sheep	Amount mgm./hr.	Amount gm./day	No. of observations	No. of sheep
Anæsthetized Mean Range	151·5 (84·0–207)	3·64 (2·02–4·97)	7	3	31·4 (19·1–47·2)	0·75 (0·46–1·13)	54	3
Conscious—not eating Mean Range	258·6 (31·2–1,020·0)	6·21 (0·75–24·48)	14	6	19·5 (2·4–76·7)	0·47 (0·06–1·84)	15	6
Mean—all data	227·7	5·35			28·8	0·69		

Estimates of the volume of mixed saliva put out per day by anæsthetized and conscious sheep, and of the daily salivary nitrogen input to the reticulo-rumen of these animals are shown in Table 3. The estimate for total daily saliva output by conscious sheep agrees substantially with Somers's[7] estimate of 5 l. (range 3–6 l.). Whereas Somers[7] has indicated a mean daily saliva nitrogen input to the reticulo-rumen of 0·7–0·9 gm. for conscious sheep, I have found a lower value (mean 0·47 gm./day, range, 0·06–1·84 gm. day), but I have obtained close agreement with Somers's estimate[7] for conscious sheep with his value of 0·75 gm./day for anæsthetized animals.

The negative relationship between rate of saliva flow and saliva nitrogen previously discussed is apparent in these mean values for anæsthetized and conscious sheep. This relationship suggests that despite considerable increase in the secretion of mixed saliva, such as during feeding, the total amount of saliva nitrogen entering the rumen with the ingested feed is not greatly increased.

Thanks are due to Dr. D. F. Stewart, associate chief of the Division of Animal Health, Commonwealth Scientific and Industrial Research Organization, McMaster Laboratory, Sydney, for making available animal house facilities. Particular thanks are due to Mr. H. Lahoud, laboratory attendant, and Mr. S. A. S. Douglas of the School of Wool Technology for chemical analyses of some of these samples.

[1] Denton, D. A., *J. Physiol.*, **131**, 516 (1956).
[2] Bath, D. L., Weir, W. C., and Torell, D. T., *J. Anim. Sci.*, **15**, 1166 (1956).
[3] McManus, W. R., Ph.D. thesis, University of New South Wales (1960).
[4] Phillipson, A. T., and Mangan, J. L., *New Zealand J. Agric. Res.*, **2**, 990 (1959).
[5] Somers, M., *Austral. J. Exp. Biol. Med. Sci.*, **39** (2), 111 (1960).
[6] Somers, M., *Austral. J. Exp. Biol. Med. Sci.*, **39** (2), 133 (1960).
[7] Somers, M., *Austral. Vet. J.*, **33**, 297 (1957).

DISSOCIATION OF FOOT-AND-MOUTH DISEASE VIRUS INTO ITS NUCLEIC ACID AND PROTEIN COMPONENTS

By F. BROWN and B. CARTWRIGHT

Research Institute (Animal Virus Diseases), Pirbright, Surrey

It is well established that preparations of many different animal viruses contain smaller particles in association with the infective virus particle. Included in this group are the viruses of influenza[1], rabies[2], poliomyelitis[3], Rift Valley fever[4], African horse sickness[5] and foot-and-mouth disease[6]. In the latter case it has also been shown that the larger 25-mμ infective particle can be dissociated into the 7-mμ particle by lowering the *p*H to about 6 (ref. 7) or by heating at 56° C. (refs. 8 and 9). Recent work by Mussgay[10] showed that lowering the *p*H resulted in the release of the infective ribonucleic acid core of the virus, and Bachrach[11] has since demonstrated that a similar release of the infective ribonucleic acid occurs when the virus is heated at 56° C. The latter author, however, was unable to confirm Mussgay's findings with acidified virus[12]. The present experiments were carried out in an attempt to resolve the differences between the results of these workers, as their findings have an important bearing on the structure of the infective particle.

The infective ribonucleic acid which can be extracted with phenol from foot-and-mouth disease virus is extremely sensitive to environmental ribonuclease[12,13]. In experiments designed to show the presence of the infective ribonucleic acid, therefore, it is necessary for the enzyme to be removed from the viral suspensions or for its action to be completely inhibited. In most of the experiments described here, vesicular fluid obtained from guinea pigs infected with strain 1 (immunological type *O*) was used as the source of virus. This was partly freed from ribonuclease by three successive precipitations with half-saturated ammonium sulphate. The precipitated virus was then re-suspended at 1/10 of its initial concentration in 0·1 per cent sodium dodecyl sulphate, which was a concentration sufficient to protect infective viral ribonucleic acid from the action of the ribonuclease remaining in the virus suspension. Lowering the *p*H of this suspension to 5·6 led to a considerable reduction in virus titre (measured by intraperitoneal inoculation of 7-day-old mice). As much infective ribonucleic acid could be extracted with phenol from this acidified virus, however, as from the initial virus (Table 1). Infective ribonucleic acid was estimated by intracerebral inoculation of mice, as it is non-infective when administered intraperitoneally.

Evidence that acidification had caused breakdown of the virus into smaller particles was obtained

Table 1. Virus and Infective Ribonucleic Acid Content of Ultracentrifuge Fractions of Foot-and-Mouth Disease Virus before and after Acidification

	Virus titre in mice (log ID_{50}/ml.)*	Ribonucleic acid titre in mice (log ID_{50}/ml.)†	Complement-fixing activity‡
Initial virus diluted 1 to 10 in 0·1 per cent sodium dodecyl sulphate	7·2	3·2	94·2
Top 5 ml.	5·0	1·6	17·8
Bottom 5 ml.	7·6	3·4	72·4
Acidified virus (*p*H 5·6)	2·6	3·4	117·7
Top 5 ml.	—	3·5	56·7
Bottom 5 ml.	—	3·2	61·0

* Intraperitoneal inoculation of 0·03 ml.
† Intracerebral inoculation of 0·03 ml.
‡ Millilitres of 1/30 complement fixed by total 1/10 initial virus or fraction.

Table 2. Infectivity of Acidified Foot-and-Mouth Disease Virus by Various Routes of Inoculation

	Titre in mice (log ID_{50}/ml.)		Ribonucleic acid titre in mice (log ID_{50}/ml.)
	Intra-peritoneal	Intra-cerebral	Intra-cerebral
Initial virus diluted 1 to 10 in 0·1 per cent sodium dodecyl sulphate	7·3	7·8	3·2
Same + 100 μgm. ribonuclease per ml.	7·3	7·6	—
Acidified virus (*p*H 5·0)	2·0	3·7	3·4
Same + 100 μgm. ribonuclease per ml.	1·8	1·7	—

by centrifuging the control and acidified virus suspensions at 30,000 r.p.m. for 1 hr. in the *L*40 head of the Spinco ultracentrifuge. The upper and lower 5-ml. fractions from each tube were then separated and titrated for virus content and also extracted with phenol and the infective ribonucleic acid estimated. The results in Table 1 show that more than 90 per cent of the infective ribonucleic acid which could be extracted from the control virus was present, as virus, in the lower 5-ml. fraction. In contrast, equal amounts of infective ribonucleic acid could be extracted from the upper and lower 5-ml. fractions of the acidified virus, indicating that the entity from which the infective ribonucleic acid was extracted had a smaller sedimentation constant than the initial virus. Complement-fixation tests on the fractions showed that this activity also was distributed equally between the two fractions of the acidified virus, confirming the previous finding of Randrup[7] that the virus had disrupted to yield smaller complement-fixing particles. Unacidified virus preparations contained only 20 per cent of the initial complement-fixing activity in the top 5 ml. when centrifuged under the same conditions.

Although these results showed that acidified virus suspensions contained a fragment which was smaller than the initial virus but which still yielded infective ribonucleic acid on extraction with phenol, they could not be taken as conclusive evidence that the infective ribonucleic acid itself had been released from the virus particle. Some evidence has been obtained, however, which makes this seem likely. By lowering the *p*H of the virus suspension in 0·1 per cent sodium dodecyl sulphate to 5·0, a product was obtained which was of low infectivity when tested intraperitoneally in mice (Table 2). This suspension was, however, about fifty times more infective when inoculated intracerebrally, but all this extra infectivity could be destroyed by adding 100 μgm. ribonuclease per ml. Moreover, the infectivity of the acidified virus, tested intracerebrally, was as high as that of the ribonucleic acid which could be obtained from it by phenol extraction. It appears likely, therefore, that the infective material which was left after acidification was mainly the viral ribonucleic acid itself.

Some of these experiments have been repeated with a foot-and-mouth disease virus of type *C* (strain 997) grown in tissue culture. In these experiments also it was possible to extract infective ribonucleic acid from acidified virus of low virus infectivity. These results thus confirm the earlier work of Mussgay[10] and also show that the dissociation of foot-and-mouth disease virus into its nucleic acid and protein components is not confined to one type of the virus.

[1] Hoyle, L., Reed, R., and Astbury, W. T., *Nature*, **171**, 256 (1953).
[2] Polson, A., and Wessels, P. O., *Proc. Soc. Exp. Biol. Med.*, **84**, 317 (1953).
[3] Seizer, G., and Polson, A., *Biochim. Biophys. Acta*, **15**, 251 (1954).
[4] du T. Naude, W., Madsen, T., and Polson, A., *Nature*, **173**, 1051 (1954).
[5] Polson, A., and Madsen, T., *Biochim. Biophys. Acta*, **14**, 366 (1954).
[6] Bradish, C. J., Brooksby, J. B., Dillon, J. F., and Norambuena, M., *Proc. Roy. Soc.*, B, **140**, 107 (1952).
[7] Randrup, A., *Acta Pathol. Microbiol. Scand.*, **34**, 366 (1954).
[8] Brooksby, J. B., *Symp. Nature of Virus Multiplication: Soc. Gen. Microbiol.*, 246 (1953).
[9] Brown, F., and Crick, J., *Virology*, **5**, 133 (1958).
[10] Mussgay, M., *Monatsh. Tierheilk.*, **11**, 185 (1959).
[11] Bachrach, H. L., *Proc. Soc. Exp. Biol. Med.*, **107**, 610 (1961).
[12] Bachrach, H. L., *Virology*, **12**, 258 (1960).
[13] Brown, F., and Stewart, D. L., *Virology*, **7**, 408 (1959).

ANTITHROMBIN ACTIVITY OF NORMAL AND PATHOLOGICAL CEREBROSPINAL FLUID

By Dr. H. A. GAERTNER*, J. LISIEWICZ and Dr. J. CAEN

Blood Coagulation Laboratory, Third Medical Clinic, Krakow, and Institute for Research in Blood Diseases and Leukæmia, Claude-Bernard Association, Saint-Louis Hospital, Paris

THE coagulant activity of cerebrospinal fluid has been ascribed either to the presence of a thromboplastin[1–4] or of a factor-VIII-like[5] activity. Some authors[2–4,6] admitted that cerebrospinal fluid may contain small amounts of prothrombin. Normal cerebrospinal fluid contains neither fibrinogen nor fibrin; they appear in pathological cerebrospinal fluid when its protein-level increases beyond 100 mgm. per cent[7].

Other investigators demonstrated the anticoagulant properties of cerebrospinal fluid. In idiopathic hæmorrhage the red clot does not form from the extravasated blood in the presence of cerebrospinal fluid[8]. The fibrinogen of tuberculous cerebrospinal fluid clots more slowly than the fibrinogen of the

* Fellow of the Rockefeller Foundation.

blood[9]. Gohr and Scholl[1] found in cerebrospinal fluid an inhibitor of thrombin generation which has no effect on the already generated thrombin. They denied any identity between this inhibitor and heparin, while Kafka[2-4] denied the existence of an antithrombin in cerebrospinal fluid at all.

In spite of the last negative statements we decided to re-investigate the problem of the antithrombin activity of normal and pathological cerebrospinal fluid.

(1) The cerebrospinal fluid was collected by lumbar puncture and centrifuged for 10 min. at 3,000 r.p.m. in order to sediment and eliminate the morphological elements. The determinations were performed after storing cerebrospinal fluid for 6 hr. at 4° C. (2) The primary solution of thrombin ('Thrombofort'—Richter plus distilled water) was diluted with veronal–acetate buffer in equal volumes in order to obtain a secondary solution. Only a part of the primary solution was used to prepare the secondary one, which allowed us to standardize the activity of the first thrombin solution. 0·2 ml. of the secondary solution must clot 0·2 ml. of oxalated deprothrombinized plasma within 30 ± 2 sec. (3) Deprothrombinized plasma was prepared from a pooled oxalated plasma from at least five blood-donors by shaking for 10 min. with barium sulphate (1 : 10) and subsequent centrifugation. This plasma, free from factors II, VII, IX and X, and containing factors V, VIII and XI (plasma thromboplastin antecedent), did not clot for at least 15 min. after the addition of equal volumes of thromboplastin and *M*/40 solution of calcium chloride.

To determine thrombin inactivation 0·4 ml. of the primary thrombin solution was mixed with 0·4 ml. of cerebrospinal fluid. Immediately, 0·2 ml. of this mixture was added to 0·2 ml. of oxalated deprothrombinized plasma. The coagulation time of the mixture was determined. The determination was carried out again after a 10- and 20-min. incubation of thrombin solution with cerebrospinal fluid. In a control reaction 0·2 ml. of veronal–acetate buffer (*p*H 7·35) was used instead of cerebrospinal fluid. All determinations were performed at room temperature.

The first group of experiments concerned twenty specimens of fluid showing no pathological changes in the routine laboratory tests (protein, globulin, sugar and chloride levels, pleocytosis, Nonne-Apelt, Weichbrodt and Pandy reactions). Table 1 shows that after 10- and 20-min. incubation the thrombin activity of the experimental mixture decreases slowly and progressively in the presence of normal cerebrospinal fluid. A certain tendency to a greater prolongation of the coagulation time in the presence of a higher concentration of proteins appeared, but was neither stable and repeatable nor statistically significant. The comparative investigations showed that the antithrombin of normal cerebrospinal fluid is considerably weaker than the antithrombin of blood and of pathological cerebrospinal fluid. Toluidine blue (0·05 ml. of a 2·5 mgm./ml. concentration) and protamine sulphate (0·05 ml. of a 1 mgm./ml. concentration) reduced, but did not abolish, the antithrombin activity of cerebrospinal fluid as compared with control reactions with distilled water (0·05 ml.). The antithrombin activity of normal cerebrospinal fluid did not change after adsorption on barium sulphate, but disappeared after addition of ether. Heating for 1 hr. at 45° C. did not influence essentially the antithrombin activity of normal cerebrospinal fluid. After 10 min. at 75° and 1 min. at 100° the antithrombin activity of normal cerebrospinal fluid disappeared.

Table 1

	No. of cases	Time for incubation (min.) 0		10		20	
Normal cerebrospinal fluid	20	29·75	(30)*	33·55	(30)	37·37	(31)
Normal serum	10	27·4	(30)	95·5	(30)	205·6	(31)
Toluidine blue	10	27·6	(29·6)	30·4	(34·4)	33·6	(39·4)
Protamine sulphate	10	27·1	(29·7)	30·6	(34·4)	33·9	(38·5)
Barium sulphate	10	29·45	(29·1)	32·85	(33·3)	36·1	(38·2)
	5†	*29·6*	*(29·5)*	*37·6*	*(70·2)*	*42·4*	*(120·6)*
Ether	5	29·2	(29·8)	28·5	(34·3)	30·8	(38·5)
75°; 5 min.	5	29·4	(29·2)	31·2	(33·4)	32	(38)

The mean values of the residual uninactivated thrombin time are given in seconds.
* Mean values of control determinations (in brackets).
† Normal cerebrospinal fluid in ordinary type; pathological fluid in italics.

Table 2

Diagnosis	Incubation time (min.) 0	10	20	Proteins mgm. per cent	Globulins mgm. per cent	Pleocytosis	Nonne-Apelt, Weichbrodt and Pandy tests
1. Tuberculous meningitis	30	170	308	210	0·06	312	+
2. ,, ,,	27	43	60	45	0·04	37	+
3. ,, ,,	28	36	48	30	0·03	42	+
4. ,, ,,	29·5	66	143	30	0·03	31	+
5. ,, ,,	30	56	126	30	0·03	24	+
6. Pneumococcal meningitis	29	34	41	85	—	40	+
7. ,, ,,	31*	165	623	480	—	1,690	+
	29	52	99	300	—	479	+
	29	44	73	—	—	240	+
	30·5	43	56	160	—	115	+
8. Meningitis	26	32·5	40	60	—	8/3	+
9. Encephalitis and meningitis	32	38	45	105	0·1	98	+
10. ,, ,,	29	33	38	55	—	13/3	+
11. Hydrocephalus	26	42	64	240	—	25	+
	25·5†	29	41	320	—	'Many'	+
12. Progressive palsy	30	37	45	76	0·2	243	+

The values of the residual uninactivated thrombin time are given in seconds.
* Determinations of October 14, 17, 19, and 24, 1960.
† Ventricular cerebrospinal fluid.

The second group of experiments concerned twelve specimens of fluid with abnormal results in the laboratory tests (Table 2). The inactivation of thrombin in pathological fluid is greater than in normal fluid and sometimes even greater than in serum. The results show no correlation between the protein-level or pleocytosis and antithrombin activity of the pathological cerebrospinal fluid. In one case of pneumococcal meningitis the antithrombin activity decreased in parallel to the improvement of clinical signs and laboratory tests. In a patient with hydrocephaly the cerebrospinal fluid obtained by ventricular puncture showed a lower antithrombin activity than the fluid obtained by lumbar puncture. The pathological antithrombin activity disappears after adsorption on barium sulphate and after 10 min. heating at 75° (Table 1).

Our results provide evidence for the existence of an antithrombin activity in normal and pathological cerebrospinal fluid. We have been able to determine some of their physico-chemical and biological properties which allowed us to differentiate between the normal and pathological antithrombins of cerebrospinal fluid and the blood antithrombins (Table 3).

The antithrombin of normal cerebrospinal fluid has a progressive action considerably weaker than that of the blood antithrombin or of pathological fluid. Toluidine blue and protamine sulphate reduce

Table 3*

Nature	Action	Influence of ether	Adsorption on barium sulphate	Thermo-stable	Thermo-labile	Neutralization by toluidine blue and protamine sulphate	Generation during fibrino- or proteo-lysis
I. Fibrin	Immediate	–	–	4°; 1 (month)	56°; 10 (min.)	–	–
Heparin	Immediate	–	+	4°; 1 (month) 100°; 10 (min.)		+	–
II. Lipoprotein	Immediate	+	–	4°; 1 (month) 56°; 10 (min.)	60°; 10 (min.)	+	–
III. Lipoprotein	Progressive	+	–	4°; 1 (month) 56°; 10 (min.)	60°; 10 (min.)	–	–
IV.	Immediate	–	–	60°; 10 (min.)	70°; 10 (min.)	–	–
V. Euglobulin	Immediate	–	–	56°; 10 (min.)	100°; 10 (min.)	–	–
VI. Fibrinopeptide	Immediate	–	–		60°; 30 (min.)	Partially	+
Brain antithrombin (lipid?)	Immediate	+		4°; 1 (month) 60°; 20 (min.) 100°; 10 (min.)		–	–
Normal cerebrospinal fluid antithrombin (lipoprotein?)	Progressive	+	–	45°; 60 (min.)	75°; 5 (min.) 100°; 1 (min.)	Partially (?)	–
Pathological fluid antithrombin	Progressive		+		75°; 5 (min.) (?)		?

* Refs. 11–15.

but do not destroy the antithrombin activity of normal cerebrospinal fluid. This activity is not adsorbed by barium sulphate or destroyed by heating (45°, 1 hr.), but disappears after addition of ether or heating at 75° for 10 min. and at 100° for 1 min.

The considerable antithrombin activity of the pathological cerebrospinal fluid is due either to the increase of physiological antithrombin or to the appearance of an abnormal antithrombin(s ?). The last possibility is even more probable because the pathological antithrombin is adsorbed on barium sulphate which does not adsorb the physiological antithrombin of cerebrospinal fluid. The pathological antithrombin activity seems to be higher in the acute phase of inflammatory (meningitis) than in chronic neurological diseases (especially degenerative diseases, for example, progressive palsy) and to decrease with improvement of clinical and laboratory signs. The relationship between the antithrombin of pathological cerebrospinal fluid and antithrombin I and VI, trypsin (present in inflammatory cerebrospinal fluid)[10] or other proteo-fibrino(geno)lytic agents can be established only after further investigations. Contrary to the behaviour of blood antithrombins, the pathological antithrombin of cerebrospinal fluid is adsorbed on barium sulphate and has a progressive action (Table 3).

The question as to where the antithrombin of normal and pathological cerebrospinal fluid is formed and how it enters the fluid cannot be answered. Also the significance of, and part played by, the normal and pathological antithrombin of cerebrospinal fluid remain unknown. The investigations on the hæmostatic (especially antithrombin) activity of normal and pathological fluid can possibly help the diagnosis, differentiation and prognosis of certain neurological diseases.

The investigations were supported by the Rockefeller Foundation and by the Polish Institute of Sciences in the United States.

[1] Gohr, H., and Scholl, O., *Deutsches Gesundheitswesen*, **8**, 1336 (1953)
[2] Kafka, V., *Nervenarzt*, **26**, 166 (1955).
[3] Kafka, V., *Acta Psychiatr. Neurol.*, **25**, 67 (1950).
[4] Kafka, V., *Acta Med. Scand.*, **120**, 146 (1954).
[5] Kundu, H. D., and Chatterjea, J. B., *Bull. Calcutta School Trop. Med.*, **9**, 57 (1961).
[6] Kotschy, A., quoted in ref. 2.
[7] Kabat, E. A., Moore, D. H., and Landow, H., *J. Clin. Invest.*, **21**, 571 (1942).
[8] Dowzenko, A., *Polski Tygodnik Lekarski*, **38**, 1162 (1946).
[9] Ruska, H., and Wolpers, C., *Klin. Wschr.*, **19**, 695 (1940).
[10] Kaplan, J., Cohn, D. J., Lewinson, A., and Stern, B., *J. Lab. Clin. Med.*, **25**, 495 (1940).
[11] Gaertner, H. A., *Coagulation of the Blood*, Polish text, 528 (Krakow, 1960).
[12] Masure, R., *Les inhibiteurs normaux et pathologiques de la coagulation sanguine*, 284 (Arscia, Brussels and Masson, Paris, 1960).
[13] Quick, A. J., *Hemorrhagic Diseases*, 451 (Lea and Febiger, Philadelphia, 1959).
[14] Seegers, W. H., *Blood Coagulation—Selected Papers 1953–1957*, 530 (Detroit, 1957).
[15] Soulier, J. P., *Le Sang*, **30**, 262 (1959).

EFFECTS OF ELECTRICAL STIMULATION OF RETICULAR FORMATION ON THE LACTIC ACID CONTENT OF THE RAT BRAIN

By Lj. MIHAILOVIĆ, Lj. KRŽALIĆ and D. CVETKOVIĆ

Institute of Pathological Physiology, Medical School, University of Belgrade

IT is customary, especially in the literature on electrophysiology, to speak about the 'activating', 'energizing', or 'dynamogenic' action of the brain-stem reticular formation on the electrical activity of the cerebral cortex. Since the pioneer work of Moruzzi and Magoun[1], a great deal of information has been accumulated concerning the electrical manifestations of this activating process and its neuronal mechanism. However, little is known about the metabolical correlates of this activation, that is, about the biochemical events triggered by impulses from reticular neurones. It is generally assumed that any form of stimulation or departure from the resting state might start glycolytic processes, with the liberation of inorganic phosphorus and lactic acid. It has been reported that the metabolical activity of the brain is increased in trauma, shock, convulsions and emotional excitement and reduced in anæsthesia and sleep[2].

Starting from these investigations and the well-established role of the reticular formation in the regulation of the states of wakefulness and sleep, the present investigation was carried out to find out if similar biochemical changes take place in the brain following electrical stimulation of the brain-stem reticular formation and if they could be correlated with concomitant changes in electrical activity.

Young rats of 50–90 gm. anæsthetized with 'Nembutal' (50 mgm./kgm. body-weight) were used in the experiments. Recording electrodes were placed on the frontal and parietal cortex. A fine stimulating electrode (0·01 in. diameter) was inserted stereotaxically in the brain-stem reticular formation according to co-ordinates based on measurements previously made on several animals of similar age and size. The operative procedure was completed in 10 min. after initiation of anæsthesia in all experiments. Stimulating and recording was programmed in such a way as to permit immersion of the animals into liquid air exactly at the end of the twentieth minute after 'Nembutal' injection. Thus, the period of anæsthesia was maintained constant in all the experiments. Electrical stimuli (0·3 m.amp., 300 cycles/sec. and 0·25 m.sec. pulse duration), delivered by a Grass stimulator model 4*S*, were given for two periods of 10 sec., with an interval of 5 sec. between the two stimulations. The electrocorticogram was recorded throughout the experiment using a Reega Alvar apparatus. This provided an index of the blood and oxygen supply to the brain during the experiment. Details of the stimulation and recording technique are published in ref. 3.

Rats were divided into eight groups (each consisting of at least seven animals). Rats from the first (control) group received only an injection of 'Nembutal'; those from the second (control) group were placed in the stereotaxic instrument, incision was made and holes drilled in the skull; the third (control) group consisted of rats, with cortical and reticular electrodes inserted, which were not subjected to stimulation. Animals belonging to the fifth, sixth, seventh and eighth group were stimulated as described and dropped in the liquid air 30 sec., 1 min., 3 and 5 min. respectively, after cessation of electrical stimulation. In order to secure rapid fixation of biochemical changes and prevent post-mortem glycolysis, animals were killed by immersion in the liquid air. Reasons for freezing the whole animal have been described (ref. 4). To facilitate dissection, the frozen animals were immediately transferred into a deep-freezer at − 20° C. Rapidly dissected with cooled instruments, brains (sectioned just in front of the cerebellum) were dropped in the liquid air and powdered in a previously cooled crusher. Lactic acid was estimated according to the method of Friedemann *et al.* with modifications introduced by other authors[5].

The results are illustrated in Fig. 1. In the first (control) group of animals, killed after being in anæsthesia for 20 min., the lactic acid content ranged from 6·5 to 10·5 mgm. per cent with a mean of 8·9 (broken line in Fig. 1). These figures are in agreement with values obtained by other authors under similar conditions[4]. Estimations of the lactic acid content of the brain of rats comprising the second and third (control) group of animals gave somewhat higher values than those of the first group (12·0 and 12·5 mgm. per cent respectively—full line in Fig. 1). This showed that fixing the animal into the stereotaxic apparatus and the operative procedure used, already raised to some extent the lactic acid content of the brain. It is conceivable, therefore, that this level should be taken as a basis for comparing values obtained after electrical stimulation. As seen in Fig. 1, electrical stimulation of brain stem reticular formation was consistently found to be followed by a rapid and significant increase of the lactic acid content of the brain ($P > 0.001$ with Fisher's t test). No such high values were obtained following focal stimulation of cerebral cortex under identical conditions or even after strong hippocampal after-discharge produced by insertion of electrode into this structure. Experiments in which the period between the cessation of electrical stimulation and immersion of animal into the liquid air was varied from 30 sec. to 5 min. (Fig. 1) showed that this increase was of short duration, followed by a return of the lactic acid within the limits of control variations 5 min. after discontinuing the stimulation.

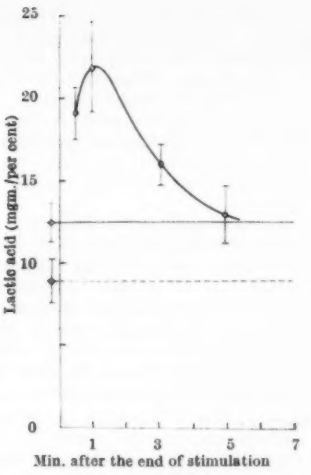


Fig. 1

It should be pointed out that raising the lactic acid outlasted the transient desynchronization of the electrocorticogram. When increase of lactic acid was at a peak (that is, between 30 sec. and 1 min. after discontinuing the stimulation) the pre-stimulation type of electrical activity was usually recorded.

Formation of the lactic acid in the brain under normal physiological conditions has been demonstrated by Gibbs *et al.* Experiments of Richter and Dawson showing that the lactic acid content of the brain is lowered in normal sleep and increased in emotional excitement provided further evidence that the rate of lactic acid formation varies with the functional activity of the brain[4]. In another series of experiments these authors reported that electrical stimulation of the brain produces a rapid fall in the phosphocreatine content and transient rise in the hexose phosphate fraction followed by their return to normal level 15–45 sec. after electrical stimulation, indicating the dynamic balance between the breakdown and re-synthesis of high-energy phosphate bonds[7].

Results reported in the present article also suggest that biochemical events, underlying the 'dynamogenesis', or increased functional activity of the brain

induced by electrical stimulation of reticular formation, involve (at least as a component of a more complex mechanism) the breakdown of high-energy phosphorus compounds. Triggered by the impulses generated by reticular neurones it sets in motion the processes leading to lactic acid formation. Our results confirmed the finding of previous workers[4] that the brain lactic acid does not rapidly disappear from the tissue when once it has been formed.

From these considerations it follows that, unless it is believed that changes produced by electrical stimulation of brain stem reticular formation in our experiments are not of a physiological nature, it must be inferred that physiological processes resulting from an increased functional activity of reticular neurones (such as arousal or increased attention) are associated with increased glycolytic activity and the liberation of the lactic acid in the brain.

We thank Dr. R. M. C. Dawson, Institute of the Department of Animal Physiology, Cambridge, for kind advice regarding the method of lactic acid estimation.

This work was supported in part by a grant from the Yugoslav Foundation for Scientific Research, contract No. 490/1.

[1] Moruzzi, G., and Magoun, H. W., *EEG Clin. Neurophysiol.*, **1**, 455 (1949).
[2] Stone, W. E., *Biochem. J.*, **32**, 1908 (1938). Le Page, G. A., *Amer. J. Physiol.*, **146**, 267 (1946). Richter, D., and Dawson, R. M. C., *Nature*, **161**, 205 (1948). Le Page, G. A., *Amer. J. Physiol.*, **147**, 446 (1946).
[3] Mihailović, Lj., and Delgado, J. M. R., *J. Neurophysiol.*, **19**, 21 (1956).
[4] Richter, D., and Dawson, R. M. C., *Amer. J. Physiol.*, **154**, 73 (1948).
[5] Friedemann, N. R., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, **73**, 335 (1927). Friedemann, T. E., and Graeser, J. B., *ibid.*, **100**, 291 (1933). Edwards, H. T., *ibid.*, **125**, 571 (1938). Stone, W. E., *Biochem. J.*, **32**, 1908 (1938).
[6] Gibbs, E. L., Lennox, W. G., Nims, L. F., and Gibbs, F. A., *J. Biol. Chem.*, **144**, 325 (1942).
[7] Dawson, R. M. C., and Richter, D., *Amer. J. Physiol.*, **160**, 203 (1950).

BIOSYNTHESIS OF CAROTENOIDS IN PURPLE BACTERIA: A RE-EVALUATION BASED ON CONSIDERATIONS OF CHEMICAL STRUCTURE

By SYNNOVE LIAAEN JENSEN, Dr. GERMAINE COHEN-BAZIRE and Prof. R. Y. STANIER

Institute of Organic Chemistry, Technical University of Norway, Trondheim, and Department of Bacteriology, University of California, Berkeley, California

THE photosynthetic apparatus of purple bacteria contains a distinctive array of carotenoid pigments: they are aliphatic, and mostly bear methoxyl groups. Five main pigments, together with their monohydroxy derivatives, have been found in the species so far examined: lycopene, *P*481 (anhydrorhodovibrin), spirilloxanthin, pigment *Y* and spheroidenone (formerly known as pigment *R*). On the basis of their biological distribution, these ten pigments can be subdivided into two main groups (Table 1). Some species of purple bacteria contain exclusively pigments of the lycopene, *P*481 and spirilloxanthin types (group *A* pigments); others, exclusively those of the *Y* and spheroidenone types (group *B* pigments). These regularities in distribution in themselves strongly suggest that the pigments of groups *A* and *B* represent the products of two divergent biosynthetic pathways. Stoichiometric interconversions of pigments belonging to each group have been demonstrated to take place in certain circumstances within the bacterial cell, and this and the nature of the observed conversions establish unequivocally two sets of biosynthetic interrelationships[4–6]:

lycopene → *P*481 → spirilloxanthin; and
Y → spheroidenone

Table 1. Natural Distribution of the Major Carotenoids in Purple Bacteria

	Group *A*			Group *B*		
	Lycopene	*P*481	Spirilloxanthin	*Y*	Spheroidenone	
Rhodospirillum molischianum	+	–	–	–	–	refs. 1, 2
Rhodospirillum photometricum	+	+	–	–	–	
Rhodospirillum rubrum	+	+	+	–	–	
Rhodopseudomonas palustris	+	+	+	–	–	
Chromatium	+	+	+	–	–	ref. 3
Rhodopseudomonas spheroides	–	–	–	+	+	refs. 1, 2
Rhodopseudomonas capsulatus	(+)	–	–	+	+	
Rhodopseudomonas gelatinosa	–	–	(+?)	+	+	

In *Rhodospirillum rubrum*, synthesis of all group *A* pigments can be suppressed by bacterial growth in the presence of an appropriate concentration of diphenylamine. In these circumstances, there is a massive intracellular accumulation of carotenoids more saturated than lycopene (phytoene, phytofluene, ζ-carotene, neurosporene). After removal of the diphenylamine, these reduced carotenoids can be converted within the cell to the typical group *A* pigments. The analysis of the diphenylamine effect in *Rhodospirillum rubrum* has permitted the formulation of the following biosynthetic sequence for the group *A* pigments[4]:

phytofluene → ζ-carotene → neurosporene → lycopene / OH-lycopene →
*P*481 → OH-*P*481 → spirilloxanthin / OH-spirilloxanthin

So far, only one mutant of *Rhodospirillum rubrum* with a specific block in carotenoid synthesis has been found. The only carotenoid produced by this mutant

Table 2. Carotenoid Pigments in the Wild-Type and in 3 Mutant Classes of *Rhodopseudomonas spheroides* grown under Anaerobic Conditions in the Light

	Phytoene	Phytofluene	ζ-Carotene	Neurosporene, chloroxanthin	*Y*, OH-*Y*	Spheroidenone, OH-*R*	
Wild type	–	tr.	tr.	tr.	+	+	(refs. 8, 9, 11)
Brown mutant	–	tr.	tr.	+	+	tr.	(ref. 9)
Green mutant	–	tr.	tr.	+	–	–	(ref. 10)
Blue-green mutant	+	—	—	—	—	–	(ref. 11)

is phytoene[7]; the specific site of the enzymatic defect is thus reasonably interpreted as lying at the step between phytoene and phytofluene.

The biosynthetic origin of the group *B* pigments has been investigated in *Rhodopseudomonas spheroides*, from which a series of mutants with specific blocks in carotenoid synthesis has been isolated. The patterns of accumulation in these mutants (Table 2) are most readily interpreted in terms of the following sequence:

phytoene → phytofluene → ζ-carotene →

$$\left\{\begin{array}{l}\text{neurosporene}\\ \text{chloroxanthin}\end{array}\right\} \rightarrow Y \rightarrow \text{spheroidenone}$$

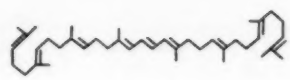
The biochemical data accordingly suggest that the pigments of groups *A* and *B* are synthesized from a common series of more saturated carotenoids originating with phytoene, the specific pathways diverging from one another subsequent to the formation of neurosporene. In support of the hypothesis of a common origin for the pigments of both groups, it should be added that traces of neurosporene, ζ-carotene and phytofluene can be found in exponentially growing cells of the wild types of both *Rhodospirillum rubrum* and *Rhodopseudomonas spheroides*[4,7].

The biological experiments which led to these interpretations of carotenoid synthesis in purple bacteria were carried out several years ago. At that time, there were many uncertainties and some misconceptions concerning the chemical structures of the compounds involved. Consequently, it was not then possible to formulate in chemical terms the postulated reaction sequences, or to establish the chemical nature of the individual step-reactions. These uncertainties and misconceptions concerning carotenoid structure have been largely eliminated by recent chemical work, which reveals that the earlier biological data in fact conform with a rather simple and chemically intelligible biosynthetic scheme. We shall consider in turn the nature of the common primary step-reactions and of the specific step-reactions which lead to the group *A* and group *B* pigments.

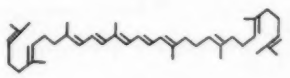
The Common Primary Step-Reactions

The structure of phytoene (I) was well established by the work of Rabourn and Quackenbush[12]. The same workers afterwards assigned structures to phytofluene (II) and ζ-carotene (III)[13,14], although the chemical evidence for these structures has not yet been published. Zechmeister[15] also recently suggested structure II for phytofluene. Structures I, II and III for these carotenoids have been confirmed in an elegant fashion by Davis, Jackman, Siddons and Weedon[16]. Neurosporene was long considered to be identical with 5,6,5′,6′-tetrahydrolycopene, synthesized by Eugster, Linner, Trivedi and Karrer[17], and thus to be a structural isomer of ζ-carotene (III). The improbability of an isomeric relationship between ζ-carotene and neurosporene in the light of the chemical evidence available had been pointed out by several authors[18,19], but definite proof that neurosporene is a dihydrolycopene with structure IV has only recently been furnished by Davis, Jackman, Siddons and Weedon[16].

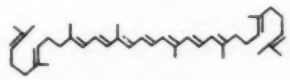
Phytoene is characterized by the possession of a centrally located chromophore with 3 conjugated double bonds, and of 6 isolated double bonds; the molecule thus possesses complete bilateral symmetry.



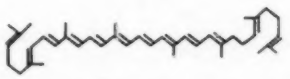
Phytoene (I)



Phytofluene (II)



ζ – carotene (III)



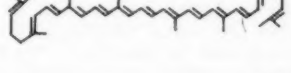
Neurosporene (IV)

The successive formation of phytofluene, ζ-carotene and neurosporene from phytoene evidently involves a series of stepwise dehydrogenations: at each step, a new double bond is introduced alternatively to the left and to the right side of the phytoene chromophore. Each double bond so introduced brings into conjugation one of the previously isolated double bonds, and the length of the chromophore is accordingly increased by two conjugated double bonds at each step. The biosynthesis of neurosporene from phytoene therefore proceeds by three successive dehydrogenations, identical in mechanism and differing from one another only in the specific points on the polyene chain at which they take place.

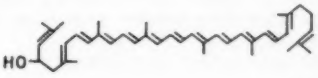
A reaction sequence of this type was in essence first proposed by Zechmeister[20], on the basis of chemical considerations, to account for the biological origin of the coloured carotenoids. This hypothesis was further elaborated by Porter and Lincoln[21], who used it to explain the genetic and biochemical data concerning carotenoid synthesis in tomato fruits. The so-called 'Porter–Lincoln sequence', which is in fact the only coherent general hypothesis ever advanced to explain the biological formation of coloured carotenoids, was long treated with scepticism by most workers in the field (see, for example, refs. 22 and 23). Such scepticism can no longer be justified.

Biosynthesis of the Group A Carotenoids

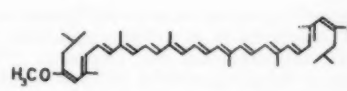
At the time when the biosynthetic pathway from lycopene to spirilloxanthin was determined in *Rhodospirillum rubrum*, the structures of three members of the sequence were assumed to be established: lycopene (V)[3], lycoxanthin (VI)[3,24] and spirilloxanthin (VII)[25]:



(V)

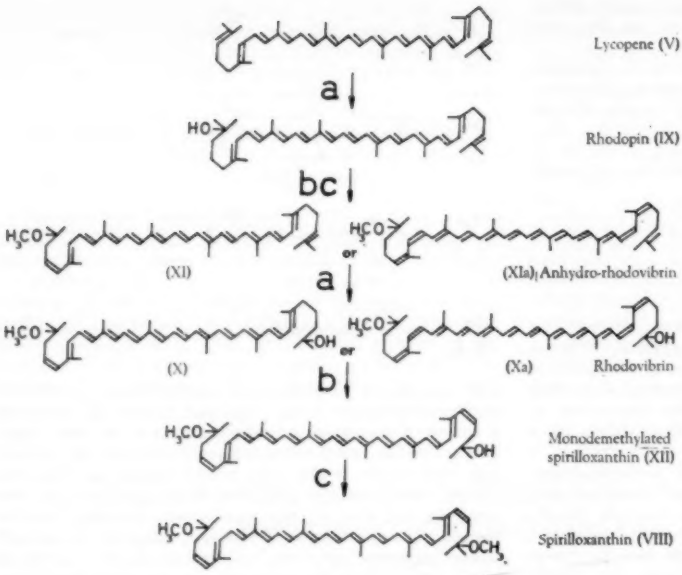


(VI)



(VII)

However, recent chemical investigations have led to a revision of the structure of spirilloxanthin



(VIII)[26–28], and to the demonstration that the monohydroxylycopene formed in *Rhodospirillum rubrum* is identical with rhodopin, not with lycoxanthin (VI). Structure IX has been assigned to rhodopin[29,30]. Furthermore, the structures of the pigments known as OH–*P*481, *P*481 and OH-spirilloxanthin have been largely determined. OH-*P*481 is identical with rhodovibrin, and has either structure X or structure X*a* (ref. 31). *P*481 (anhydro-rhodovibrin) has either structure XI or structure XI*a* (ref. 32). In the case of these two pigments, the uncertainty concerns the exact position of the chromophore in the molecule. OH-spirilloxanthin is a monodemethylated spirilloxanthin (XII) (ref. 28).

In the light of these chemical facts, a simple and consistent interpretation of the biosynthetic transformations between lycopene and spirilloxanthin can now be offered[33]. These transformations involve three different types of chemical reactions, each of which takes place twice in the overall sequence.

(*a*) The hydroxylation step: addition of water to an isopropylidene double bond, with formation of a tertiary alcohol.

(*b*) The dehydrogenation step: introduction of an additional double bond in conjugation with the already existing chromophore, either at the same side as the hydroxyl group (assuming formulæ X and XI for rhodovibrin and anhydro-rhodovibrin) or by α,ω-dehydrogenation (assuming formulæ X*a* and XI*a* for the two compounds in question). For reasons of biochemical analogy which will be shortly discussed, we prefer the first formulation and thus favour formulæ X and XI.

(*c*) The methylation step: methylation of a tertiary hydroxyl group.

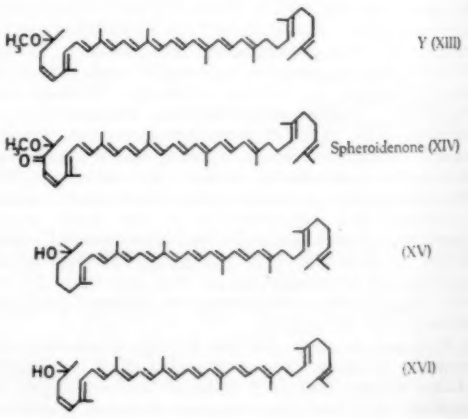
The initial reaction which leads to the formation of the group *A* pigments is a dehydrogenation: the conversion of neurosporene to lycopene. This reaction is not a dehydrogenation of type *b*, but is analogous rather to the successive dehydrogenations which result in the conversion of phytoene to neurosporene.

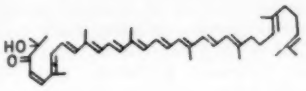
Biosynthesis of the Group *B* Carotenoids

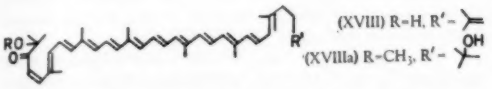
Goodwin, Land and Sissins[8], who first investigated the chemical structure of pigment *Y* and spheroidenone (pigment *R*), showed that they were monomethoxy compounds, and that spheroidenone had a conjugated keto group. The actual structures which they proposed[7,33] were, however, incorrect. Definitive structures for pigment *Y* (XIII) and spheroidenone (XIV) have recently been established by Barber, Jackman, Weedon and Yokoyama[34]. Pigment *Y* can be visualized as consisting of half a spirilloxanthin molecule (VIII) coupled with the more saturated half of the neurosporene molecule (IV). This, in turn, suggests that a sequence of reactions similar to those operative in spirilloxanthin synthesis must occur in the synthesis of pigment *Y* from neurosporene. Structurally speaking, this would require the formation of compounds XV and XVI as intermediates.

Structure XV has recently been suggested for chloroxanthin by Barber, Jackman, Weedon and Yokoyama[34]. Chloroxanthin, first characterized by Nakayama[9], is a monohydroxy derivative of neurosporene that accumulates together with neurosporene in the green mutant of *Rhodopseudomonas spheroides*, which is totally blocked in the synthesis of pigment *Y* and spheroidenone.

In the wild-type of *Rhodopseudomonas spheroides* grown anaerobically in the light, pigment *Y* and spheroidenone are always accompanied by smaller amounts of related monohydroxy compounds. The properties so far reported for OH–*Y* (refs. 7 and 9) are in accord with those which can be predicted for structure XVI. We therefore suggest that OH–*Y* is non-methylated and is the immediate biosynthetic precursor of *Y*. A similar chemical relationship can be postulated between sperоidenone and OH–*R*, in which case the latter should possess structure XVII. The limited chemical data available[7,9] are in accord with such a supposition.







When the wild-type of *Rhodopseudomonas spheroides* is cultivated under semi-aerobic conditions, its carotenoid composition changes markedly from that characteristic of anaerobic (photosynthetic) cultures[7]. Pigments *Y* and OH–*Y* virtually disappear; the major carotenoids are spheroidenone and OH–*R*, accompanied by smaller amounts of a very highly unsaturated carotenoid which has been termed *P*512 (ref. 1). The adsorptive properties reported for this carotenoid[1,9] suggest that it contains at least one hydroxyl group. The positions of the absorption maxima in visible light fit with the assumption of 12 carbon–carbon double bonds and a keto group in conjugation. The biosynthesis of *P*512 can be interpreted as resulting from the dehydrogenation of OH–*R* or spheroidenone by a reaction of the type characteristic of the phytoene–lycopene sequence, suggesting structure XVIII or XVIII*a* for this carotenoid.

We plan to investigate further the chemical structures of OH–*Y*, OH–*R* and *P*512.

Conclusions

The complete scheme which we propose for the biosynthesis of the carotenoids of the purple bacteria is shown in Fig. 1. It should be noted that in this scheme structures X and XI have been employed for rhodovibrin and anhydro-rhodovibrin, respectively, even though the chemical data now available do not exclude structures X*a* and XI*a*. The preference for X and XI rests on the fact that these structures make it possible to formulate the conversion of lycopene to anhydro-rhodovibrin, and of anhydro-rhodovibrin to spirilloxanthin, as reactions strictly analogous to the conversion of neurosporene to pigment *Y*. Furthermore, α,ω-dehydrogenations of the type which must occur in the biosynthesis of spirilloxanthin assuming structures X*a* and XI*a* are not known to occur as enzymatic reactions, whereas

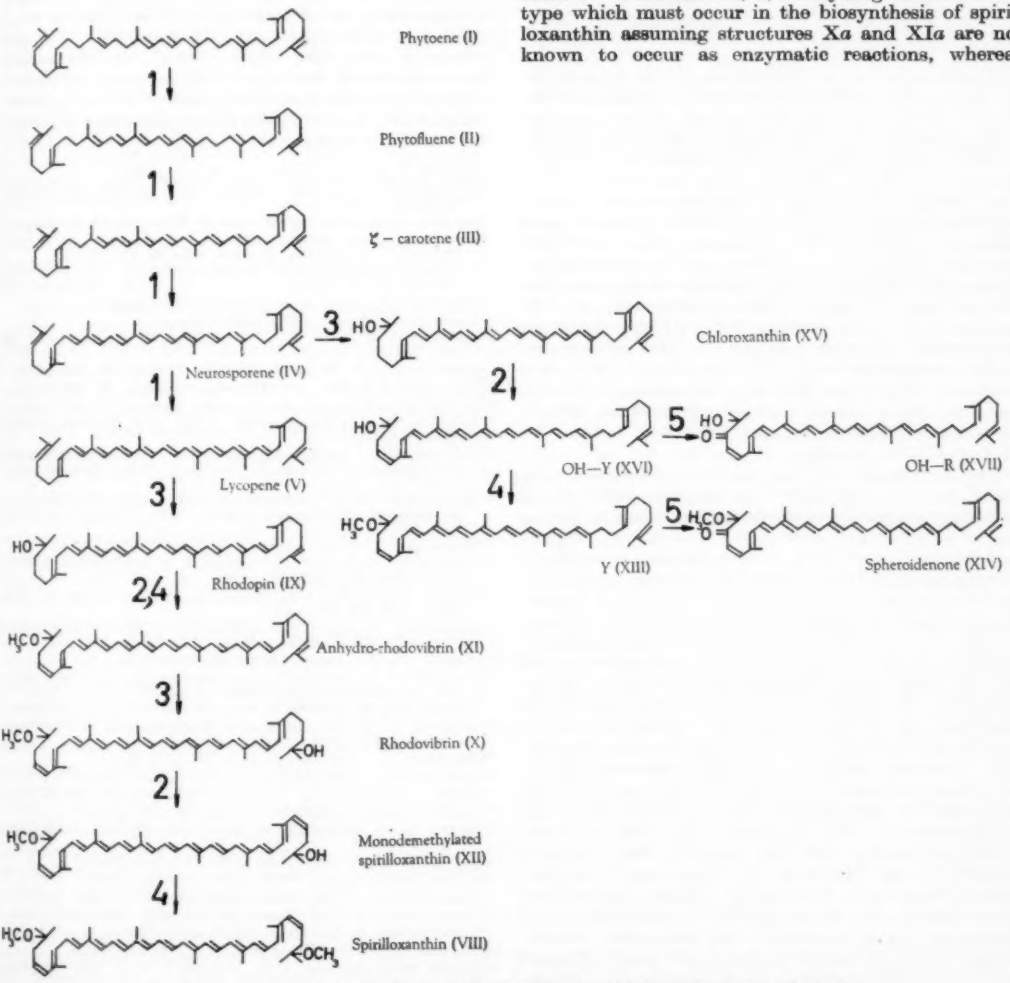
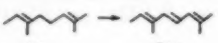
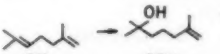
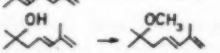
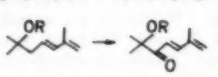


Fig. 1. Structural scheme for the pathway of carotenoid biosynthesis in purple bacteria

Table 3. The Five Types of Step-Reaction Involved in the Biosynthesis of Carotenoids in Purple Bacteria

Chemical nature	Structure of molecule at site of attack	Occurrence
1. Dehydrogenation		I→II, II→III, III→IV, IV→V
2. Dehydrogenation		IX→XI, X→XII, XV→XVI
3. Hydration		V→IX, XI(*a*)→X(*a*), IV→XV
4. Methylation		IX→XI(*a*), XII→VIII· XVI→XIII
5. Introduction of a keto group		*XIII→XIV, XVI→XVII

* R = H or CH_3

dehydrogenations across a C—C bond, like those required by structures X and XI, are exceedingly common enzymatic reactions.

The scheme outlined in Fig. 1 supposes a total of 15 step-reactions subsequent to phytoene. These 15 reactions can be grouped into five different types, depicted diagrammatically in Table 3. Each type of reaction is defined by two parameters: (*a*) the chemical nature of the change that occurs, and (*b*) the structure of the molecule immediately adjacent to the site of attack. In principle, a total of five enzymes might therefore be sufficient to catalyse all the reactions shown in Fig. 1. The biological evidence, suggests, however, that the actual number is somewhat greater. In the genus *Rhodospirillum*, *Rhodospirillum molischianum* produces only lycopene and rhodopin; *Rhodospirillum photometricum* produces lycopene, rhodopin, rhodovibrin and anhydro-rhodovibrin; and *Rhodospirillum rubrum*, all the carotenoids of the *A* group. These differences can be accounted for enzymatically only by the supposition that specific enzymes catalyse the two similar successive dehydrogenations of type 2 involved in this sequence. Both these enzymes are lacking in *Rhodospirillum molischianum*; one is present in *Rhodospirillum photometricum* and both are present in *Rhodospirillum rubrum*.

The divergence of biosynthetic pathways subsequent to the formation of neurosporene, which leads in most purple bacteria to the exclusive formation of either group *A* or group *B* pigments, implies that the dehydrogenation of neurosporene to lycopene is enzymatically distinct from the preceding dehydrogenations which result in the formation of neurosporene from phytoene, even though it also belongs to type 1. On the other hand, the accumulation patterns observed in mutants rather strongly suggest that the three dehydrogenations which result in the conversion of phytoene to neurosporene may all be catalysed by a single enzyme. Both in purple bacteria and in other organisms synthesizing carotenoids, mutants which accumulate phytoene alone have been frequently isolated. Such mutants must have lost the enzyme required to convert phytoene to phytofluene. But mutants which can be interpreted as having lost the enzyme that converts phytofluene to ζ-carotene, or ζ-carotene to neurosporene, have never been found. When a mutational block leads to the accumulation of phytofluene and ζ-carotene, these substances are invariably accompanied by much larger amounts of more oxidized carotenoids (for example, neurosporene and chloroxanthin in the green mutant of *Rhodopseudomonas spheroides*). These otherwise puzzling facts become readily intelligible on the hypothesis that all the dehydrogenations between phytoene and neurosporene are catalysed by the same enzyme, loss of this enzyme necessarily resulting in the accumulation of phytoene alone.

A full account of the chemical investigations by one of us (S. L. J.) on the structures of the group *A* carotenoids and a further discussion of the present topic will be published elsewhere.

We wish to acknowledge the kindness of Prof. B. C. L. Weedon in communicating to us the chemical structures of certain of the carotenoids discussed, prior to their publication.

Note added in proof. The biosynthetic scheme shown in Fig. 1 predicts the existence of an intermediate between rhodopin (IX) and anhydro-rhodovibrin (XI), since this conversion requires both a dehydrogenation of type 2 and a methylation. The expected intermediate, 3,4-dehydro-rhodopin, has recently been discovered by one of us (S. L. J.) in *Rhodopseudomonas palustris*. The structure of this new group *A* carotenoid has been unequivocally established by the determination of its nuclear magnetic resonance spectrum (Jackman, L. M., and Jensen, S. L., *Acta Chem. Scand.*, in the press). The position of the chromophore in 3,4-dehydro-rhodopin provides further support for the assignment of structures X and XI to rhodovibrin and anhydro-rhodovibrin, respectively.

1 Goodwin, T. W., *Arch. Mikrobiol.*, **24**, 313 (1956).
2 Goodwin, T. W., *Soc. Biol. Chemists, India*, Silver Jubilee Souvenir 1955, 271.
3 Goodwin, T. W., and Land, D. G., *Arch. Mikrobiol.*, **24**, 305 (1956).
4 Liaaen Jensen, S., Cohen-Bazire, G., Nakayama, T. O. M., and Stanier, R. Y., *Biochim. Biophys. Acta*, **29**, 477 (1958).
5 Cohen-Bazire, G., Sistrom, W. R., and Stanier, R. Y., *J. Cell. Comp. Physiol.*, **49**, 25 (1957).
6 van Niel, C. B., *Ant. van Leeuwenhoek*, **12**, 156 (1947).
7 Cohen-Bazire, G. (unpublished observations).
8 Goodwin, T. W., Land, D. G., and Sissins, M. E., *Biochem. J.*, **64**, 486 (1956).
9 Nakayama, T. O. M., *Arch. Biochem. Biophys.*, **75**, 352 (1958).
10 Nakayama, T. O. M., *Arch. Biochem. Biophys.*, **75**, 356 (1958).
11 Griffiths, M., and Stanier, R. Y., *J. Gen. Microbiol.*, **14**, 698 (1956).
12 Rabourn, W. J., and Quakenbush, F. W., *Arch. Biochem. Biophys.*, **61**, 111 (1956).
13 Rabourn, W. J., and Quackenbush, F. W., paper presented before the Division of Biological Chemistry, 132nd Meeting Amer. Chem. Soc., 1957.
14 Quackenbush, F. W., and Rabourn, W. J., paper presented before the Division of Biological Chemistry, 132nd Meeting Amer. Chem. Soc., 1957.
15 Zechmeister, L., *Prog. Chem. Org. Nat. Prod.*, **15**, 39 (1958).
16 Davis, J. B., Jackman, L. M., Siddons, P. T., and Weedon, B. C. L., *Proc. Chem. Soc.*, 261 (July 1961).
17 Eugster, C. H., Linner, E., Trivedi, A. H., and Karrer, P., *Helv. Chim. Acta*, **39**, 690 (1956).
18 Rabourn, W. J., paper presented before the Division of Biological Chemistry, 132nd Meeting Amer. Chem. Soc., 1957.
19 Winterstein, A., *Angew. Chem.*, **72**, 902 (1960).
20 Zechmeister, L., *Carotinoide. Ein biochemischer Bericht über pflanzliche und tierische Polyenfarbstoffe* (J. Springer, Berlin, 1934).
21 Porter, J. W., and Lincoln, R. W., *Arch. Biochem.*, **27**, 390 (1950).
22 Goodwin, T. W., *The Comparative Biochemistry of the Carotenoids* (Chapman and Hall, London, 1952).
23 Mackinney, G., *Ann. Rev. Biochem.*, **21**, 473 (1952).
24 Zechmeister, L., und Cholnoky, L. V., *Chem. Ber.*, **69**, 422 (1936).
25 Karrer, P., and Koenig, H., *Helv. Chim. Acta*, **23**, 460 (1940).
26 Liaaen Jensen, S., *Acta Chem. Scand.*, **13**, 381 (1959).
27 Barber, M. S., Jackman, L. M., and Weedon, B. C. L., *Proc. Chem. Soc.*, 96 (1959).
28 Liaaen Jensen, S., *Acta Chem. Scand.*, **14**, 953 (1960).
29 Liaaen Jensen, S., *Acta Chem. Scand.*, **13**, 842 (1959).
30 Liaaen Jensen, S., *Acta Chem. Scand.*, **13**, 2142 (1959).
31 Liaaen Jensen, S., *Acta Chem. Scand.*, **13**, 2143 (1959).
32 Liaaen Jensen, S., *Acta Chem. Scand.*, **15**, xl (1961).
33 Goodwin, T. W., *Qual. Plant. Mat. Veg.*, **3–4**, 262 (1958).
34 Barber, M. S., Jackman, L. M., Weedon, B. C. L., and Yokoyama, H., cf. ref. 16.

LETTERS TO THE EDITORS

GEOPHYSICS

Round-the-World Sound Waves produced by the Nuclear Explosion on October 30, 1961, and their Effect on the Ionosphere at Sodankylä

THE heavy nuclear explosion on October 30, 1961, at 8.33.33 G.M.T. at a distance of 1,160 km. in Novaya Zemlya (presumably at tropospheric heights) was recorded at Sodankylä by means of a seismograph, a microbarograph, a magnetograph, and a vertical incidence ionosonde. The deflexion of the microbarograph took place at 9.42 G.M.T. with an amplitude of about ± 1 mb. The ionosonde showed a sudden, heavy disturbance in the *E* and *F* regions at 9 G.M.T., that is, 42 min. before the deflexion of the microbarograph (Fig. 1).

On October 31, the microbarograph again showed two very distinct and strong deflexions, namely, at 18.32 and 21.28 G.M.T. These deflexions are interpreted as being caused by round-the-world waves due to the same nuclear explosion, one being propagated in the backward, the other in the forward, direction (Fig. 2). The mean velocity deduced from these round-the-world waves is 311 m./sec. and is in good agreement with calculations based on mean atmospheric conditions below the stratopause. The wave-lengths in the recorded wave-trains lie between 198 and 58 km., and the waves are supposed to have been guided in the spherical shell between the ground and the stratopause.

Because there were no pronounced magnetic effects during the ionospheric disturbance, it seemed to be clear that the latter was not caused by excessive ionization but by a shock-wave passing over Sodankylä at ionospheric heights. This shock-wave would lose its excessive velocity and be propagated later as a sound wave in the temperature minimum between the stratopause and the lower thermosphere. If the waves are long, of the same order of magnitude as those measured on the ground, and if the formula which is giving the absorption loss[1] and which is normally applied to shorter wave-lengths will hold approximately for these wave-lengths, they can be propagated around the world without excessive attenuation. On the other hand, the intensity of the returning waves in the vicinity of the explosion site is greatly increased by focusing. Therefore, it seems reasonable to assume that these waves are strong enough to cause a detectable ionospheric effect when passing over Sodankylä.

The velocity of sound waves at ionospheric heights was estimated using rocket data[2]. The mean value obtained for the velocity was 420 m./sec., which is equivalent to round-the-world travelling time of 27 hr. On the other hand, according to the estimation mentioned above, the attenuation of such a sound wave travelling 40,000 km. and having a wave-length of 200 km. does not exceed 20 db. below heights of 150 km.

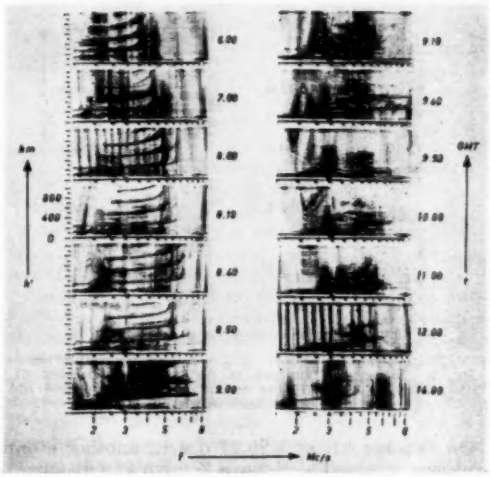


Fig. 1. Ionograms obtained at Sodankylä on October 30, 1961, showing an ionospheric disturbance caused by the nuclear explosion in Novaya Zemlya

The expected effects on the ionosphere are, indeed, to be seen on the ionograms (Fig. 3) at 9.50–11.40 G.M.T. on October 31. Although the ionograms are not completely continuous with time, we suppose that both the forward and backward propagated waves exist. This is because of the special shape of some of the oblique reflexions due to the slow sweeping speed of 4 min. over the frequency-range shown. We think that the end of the north-eastward travelling wave-train is to be seen on the ionogram at 10.50 G.M.T., whereas the end of the south-westward travelling one is to be seen on the ionogram at 11.40 G.M.T.

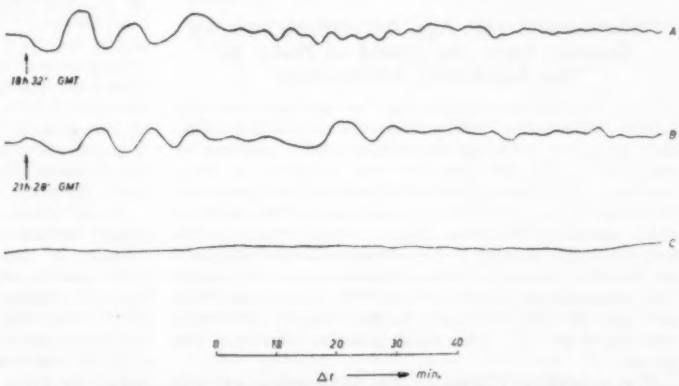


Fig. 2. Recordings of the microbarograph at Sodankylä on October 31, 1961. *A* and *B* are the deflexions of the backward and forward waves, respectively. *C* is a trace obtained under normal conditions. Pressure increases upwards

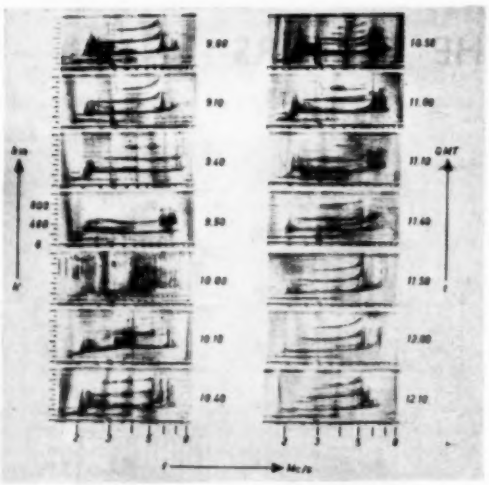


Fig. 3. Ionograms obtained at Sodankylä on October 31, 1961, showing a disturbance caused by the round-the-world ionospheric waves

On October 31, at 8.29.27 G.M.T., another atomic explosion occurred in Novaya Zemlya at a distance of 1,190 km. The deflexion of the microbarograph due to this explosion was about a third of the deflexions due to the round-the-world waves of the big explosion. Further, the ionospheric disturbance due to the latter ones was more than one hour too late to be originated from the smaller explosion. It can, therefore, be said that the smaller explosion had no detectable effect on the ionosphere.

G. Rose

Max-Planck-Institut für Aeronomie,
Lindau/Harz, Germany.

J. Oksman
E. Kataja

Geophysical Observatory,
Sodankylä,
Finland.

[1] Mitra, S. K., *The Upper Atmosphere*, second ed., 51 (Asiatic Soc., Calcutta, 1952).

[2] Newell, jun., H. E., in *Physics of the Upper Atmosphere*, edit. by Ratcliffe, J. A., 83 (Academic Press, New York and London, 1960).

Potassium-Argon Age Determinations on Granite from the Island of Mahé in the Seychelles Archipelago

The Seychelles Archipelago is situated in the Indian Ocean some 1,100 miles to the east of Mombasa. It is made up of nineteen islands and a number of islets of which all but two are granitic in composition. The only two coral islands in the group are Bird and Denis, which lie at a distance of about sixty miles north of Victoria, Mahé. Mahé itself is the largest of the islands, being seventeen miles long and up to seven miles wide. Rugged peaks of granite rise to a height of almost 3,000 ft. above sea-level and show marked erosion features. Praslin, Silhouette and Digue are the other main granitic islands of the group.

Throughout the islands, the most common rock type is pink or grey amphibolite granite. Syenite occurs on Longue Island and in the southern part of Silhouette. In various localities the plutonics are intruded by dykes of varied composition.

Sedimentary rocks are absent, though the occurrence of andalusite-hornfels on the islands of Silhouette, Cerf and Stag[1,2] may indicate that clay slates once existed.

The granitic islands lie in the centre of an extensive submarine platform that is surrounded by depths of more than 1,000 fathoms and completely separated both from the Carlsberg Ridge and from Madagascar. By any criteria other than petrographic, the islands are of oceanic type.

The age of the group is not known, but the degree of erosion suggests prolonged action by the agents of weathering. Coral fragments are to be found cemented on to the granite at heights varying between 10 and 30 ft. above low-tide level. In addition to this evidence of recent elevation, Gardiner[3] considers that a more ancient uplift of some 200 ft. occurred.

Age determinations have been carried out on samples of pink and grey granite from the island of Mahé. Owing to the small amount of biotite present in the particular samples used in this work and its partially decayed state, preliminary measurements were made on samples of the whole rock. Most of the potassium in the material is situated in the feldspars, consequently owing to loss of argon by diffusion, a total rock determination would yield an age less than the true value. However, these measurements yielded an average minimum age for the rock of 515 million years. The close agreement of the total rock ages of the pink and grey granites (Table 1) does suggest that they were formed simultaneously.

A few grams of altered biotite were separated from the pink granite in an attempt to determine its absolute age. This result is shown in Table 1 and substantiates the preliminary results.

The total-volume method was used in age determinations on the whole rock and the technique of isotope dilution used in the measurement of the biotite sample.

Owing to the coarse and variable nature of the granite it is possible that the samples used in the potassium oxide analyses of the total rock might not have been identical to those used in the argon measurements. In view of this, the combined errors arising from uncertainties in content of potassium oxide and volume of argon measurement are estimated at $\pm$ 5 per cent. Experimental errors are, however, insignificant compared with those caused by loss of argon from the feldspars. Experience has shown that 30 per cent of the argon can easily have been lost by diffusion. Though the biotite is partially altered, losses should not be of this magnitude. In Table 1 the apparent ages together with their estimated experimental errors are given. Errors arising from loss of argon cannot be accurately evaluated and have not been included.

From these measurements it is concluded that Mahé Island and most probably all the granitic islands of the Seychelles Archipelago came into existence during the very late Pre-Cambrian. Most oceanic islands are considered to be younger than Mid-Cretaceous, consequently the Seychelles are out of place chronologically and petrologically.

It is interesting to note that a large number of minerals from the continent of Africa have ages around 630 million years[4]. If the age yielded by the biotite sample is a true one, it could be evidence

Table 1. Results of Age Determinations on Granite from Mahé Island, Seychelles Archipelago

Material	Reference	Method	Volume of argon (mm.³) / Weight of sample (gm.)	K_2O (per cent)	Atmospheric contamination (per cent)	Apparent age and estimated error (m. yr.)
Grey granite, total rock	*K*/27	Total volume	0·0967	4·48	5·0	536 ± 23*
	L/6		0·0965	,,	5·1	534 ± 23*
	K/39		0·0854	,,	1·7	495 ± 22*
	L/15		0·0885	,,	3·9	501 ± 22*
	L/16		0·0941	,,	3·5	531 ± 23*
						av. = 519 ± 9*
Pink granite, total rock	*L*/9	Total volume	0·0898	4·63	3·1	497 ± 22*
	K/41		0·0918	,,	2·9	507 ± 22*
	L/23		0·0973	,,	3·8	530 ± 23*
						av. = 511 ± 10
Biotite from pink granite	*L*/29	Isotope dilution	0·1399	5·26	2·3	654 ± 17

$\lambda_\alpha = 0{\cdot}584 \times 10^{-10}$ yr.$^{-1}$.
$\lambda_\beta = 4{\cdot}72 \times 10^{-10}$ yr.$^{-1}$.
* Errors given are those arising from experimental procedure.
True errors which would take into account argon loss are much greater—see text.

suggesting an affinity between Africa and the Seychelles. This would be consistent with the view that the islands represent a continental fragment left during the widening of the Indian Ocean.

Samples of hornblende from Mahé and other islands of the Archipelago have been received from Dr. B. H. Baker of the Ministry of Commerce, Industry and Communications, Mines and Geological Department, Nairobi. Work has begun on these, and upon basaltic material from typical oceanic islands in the Atlantic.

We are especially grateful to the Governor of the Seychelles for providing the samples on which the measurements were made. Also we wish to thank Dr. D. H. Matthews of the Department of Geodesy and Geophysics, Cambridge, for his help and interest in this work, and Dr. S. O. Agrell of the Department of Mineralogy and Petrology, Cambridge, for preparing thin sections of the rocks. We are indebted to the Department of Scientific and Industrial Research for financing the project, which is part of a programme of investigation into the geochronology of oceanic rocks.

J. A. Miller
J. D. Mudie

Department of Geodesy and Geophysics,
Cambridge.

[1] Bouer, M., "Contribution to the Geology of the Seychelles, especially to the Knowledge of Laterite" (translation by the Commonwealth Bureau of Soil Science, Harpenden, Herts), *Neues Jahrbuch Mineralogie Geologie und Palæontologie*, **2**, 163 (1898).
[2] Poisson, M. C., *C.R. Acad. Sci., Paris*, (1948).
[3] Gardiner, J. S., *Nature*, **73**, 294 (1906).
[4] Holmes, A., and Cahen, L., *Colonial Geology and Mineral Resources*, **5**, No. 1, 3 (1955).

Age Determinations of Rocks in the Lizard (Cornwall) Area

A previous attempt to date the major geological events in the Lizard area[1] using the potassium–argon total volume method on biotites and muscovites gave a Caledonian age of 391 million years for the youngest intrusives, the Kennack Gneiss, but gave an anomalously younger age of 356 million years for the regionally metamorphosed Old Lizard Head Series. A series of independent measurements by Dodson[2] using the isotope dilution method gave a similar pattern with the younger age (350 million years) from muscovite from the Old Lizard Head Series and the older age (368 million years) from the Kennack Gneiss and also from biotite from the Landewednack Hornblende Schist, co-metamorphic with the Old Lizard Series. The differences between our results and those of Dodson are of the same magnitude evident in comparative measurements previously described[3].

Potassium–argon measurements have now been made on five hornblendes collected from the Kennack Gneiss (96392), the regionally metamorphosed Landewednack Hornblende Schist (96389, 96396) and the dynamothermal aureole of the peridotite (96394, 96395). The argon measurements were made by the method of isotope dilution. Potassium oxide was determined by six or eight measurements on different samples using the flame photometer. The standard deviations of these measurements are given in Table 1. Because of the low potassium content of the hornblendes the main source of error in the age values lies in the potassium oxide determinations.

The hornblende 96392 is from the same specimen of Kennack Gneiss that gave a 397-million year age from biotite. The two specimens of Landewednack Hornblende Schist are interbedded and co-metamorphic with the Old Lizard Head Series which gave an average age of 356 million years for muscovite. One hornblende (96396) gives an age agreeing with that of the Kennack Gneiss but the other (96389) gives an appreciably older age.

The two hornblendes from the metamorphic aureole of the peridotite have very different ages. No. 96395 is from the green-brown hornblende amphibolite assemblage at about 800 yards from the nearest peridotite outcrop. This gives an age (357 million years) compatible with the Caledonian ages previously described. No. 96394 is a brown hornblende from a band of hornblende–hypersthene–augite granulite immediately in contact with the peridotite. The brown hornblende gives a much greater age of 492 million years, more in harmony with the geological evidence that the regional metamorphism and peridotite intrusion are much earlier than the intrusion of the basaltic dykes and Kennack Gneiss.

The interpretation placed on these results is that the Caledonian age of 360–390 million years is a true one for the intrusion of Kennack Gneiss. This was also a period of partial or complete loss of argon for pre-existing metamorphic assemblages. The specimen of rather massive Landewednack Hornblende Schist and particularly the hornblende granulite interbanded with the peridotite at the peridotite margin may have been partly insulated from the Caledonian effects and reflect partial argon loss from Pre-Cambrian or Cambrian minerals. Hercynian thrusting may also have caused loss of argon, particularly from micas in the flat-foliated, less competent Old Lizard Head Series.

The present results are significant as further evidence that hornblendes of low potassium content can give meaningful ages in determinations by the potassium–argon method.

Table 1. Results of Potassium–Argon Dating

Material	K_2O (per cent)	Volume of argon (mm.³) / Weight of sample (gm.)	Atmospheric contamination (per cent)	δV_r	δt	Age and estimated error (m. yr.)
96392 Hornblende, Kennack Gneiss at Kennack Sands 734 E. 165 N.	0·577 ± 0·0032	0·008658	10·7	4·0	3·33	366 ± 20
96396 Hornblende, Landewednack Hornblende Schist at Pencra Head 810 E. 225 N	0·375 ± 0·0034	0·006108	16·5	2·0	3·37	371 ± 20
96389 Hornblende, Landewednack Hornblende Schist at Pen Olver 713 E. 118 N.	0·425 ± 0·0019	0·00798	12	3·8	3·94	442 ± 24
96395 Hornblende Amphibolite at Predannack Head 663 E. 160 N.	0·211 ± 0·0040	0·003768	27·2	3·0	3·25	357 ± 20
96394 Hornblende Granulite at Pol Cornick 670 E. 153 N.	0·242 ± 0·0050	0·004650	3·2	7·4	4·33	492 ± 26
Biotite from Eddystone Rock.	8·03 ± 0·0901	0·1156	4·8	0·3	3·40	375 ± 17

$\lambda\beta = 4{\cdot}72 \times 10^{-10}$ yr.$^{-1}$
$\lambda_e = 0{\cdot}584 \times 10^{-10}$ yr.$^{-1}$
δV_r = Percentage standard deviation in volume of radiogenic argon due to uncertainty in isotope ratios of argon sample.
δt = Error in age in millions of years that would be caused by a 1 per cent error in either proportion of potassium oxide or volume of radiogenic argon.
Grid references to localities refer to the National Grid.

Biotite separated from the biotite gneiss of the Eddystone Rock (obtained through the courtesy of Dr. P. A. Sabine, Geological Survey) gave a Caledonian age (375 million years) by the total volume method. This rock has in the past been generally correlated with the metamorphics of the Lizard[4]. From the experience in the Lizard it cannot be assumed that this Caledonian age is the age of crystallization of the gneiss, but it can be said that the Eddystone Gneiss forms part of the metamorphic terrain affected by the Caledonian orogenic events.

J. A. Miller
Department of Geodesy and Geophysics, Cambridge.

D. H. Green
Department of Mineralogy and Petrology, Cambridge.

[1] Miller, J. A., and Green, D. H., *Nature*, **191**, 159 (1961).
[2] Dodson, M. H., *Proc. Geol. Soc., Lond.*, No. 1591, 133 (1961).
[3] Dodson, M. H., Miller, J. A., and York, D., *Nature*, **190**, 800 (1961).
[4] Reid, C., "The Geology of the Country around Mevagissey", *Mem. Geol. Survey* (1907).

PHYSICS

Method of Measuring the Mössbauer Effect

The Mössbauer effect[1] is of great value because of the extremely high energy resolution it provides, but it is often very difficult to measure, because it may be very small (for example, about $0{\cdot}07 \times 10^{-2}$ per cent for zinc-67 in zinc metal[2]). Common values are about 1 per cent of the total absorption, and even these may be attained only at very low temperatures. A differential absorption of this order is very difficult to measure with precision, particularly at low temperatures, because the stability of the equipment must be exceptionally high. For these reasons, most of the detailed applications have involved the use of the 14·4-keV. line of iron-57, which gives about 14 per cent differential absorption at room temperature. The parent nuclide is cobalt-57, which is difficult to prepare; moreover, the internal magnetic fields in iron cause a hyperfine splitting, and the low energy (14·4 keV.) makes the radiation difficult to use. Further, very strong sources are needed, because the distance from source to counter is large and because the source is used for only a small proportion of the total time. Finally, the velocity of the source relative to the absorber varies (owing to the curvature of the path) and is known only to 10 per cent or so if a system similar to Mössbauer's original one is used.

Fig. 1 shows schematically a system designed to overcome these disadvantages. The phosphor P (of a scintillation counter) is placed inside an open-ended drum D, which bears the absorber (one, not two). The source S is mounted on a vibrating strip V. The axis of rotation of D lies below the line joining S to P, so the absorber has a known effective velocity relative to S. The drum (diameter 3 in.) is driven via a continuously variable gear; the velocity is swept evenly continuously through zero. The thick copper strip V is clamped at C and performs bending vibrations, thus causing the source to vibrate along the line S–P (a motor-driven cam provides the motion). The strip dips into liquid nitrogen. The lower edge of the drum also dips into liquid nitrogen. The alternating signal from the ratemeter is fed to a phase-sensitive detector, the output of which is the first derivative of the Mössbauer line. Fig. 2 shows a

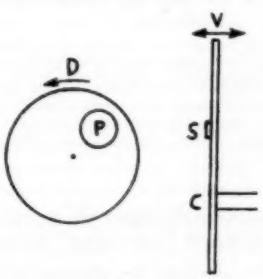


Fig. 1

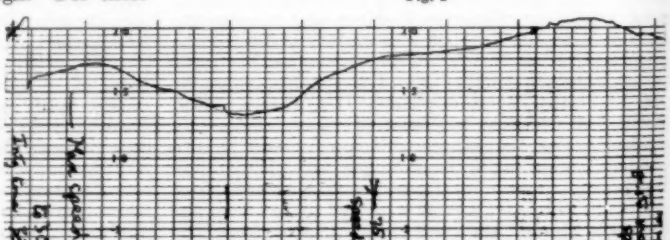


Fig. 2

recording of that derivative for the 129-keV. line of iridium 191; the source was about 60 μc., the absorber was a strip of iridium foil 5 mm. wide and 0·05 mm. thick, and the recording took about 1 hr. The entire assembly can fit into the working gap of an electromagnet.

It is hoped to publish a more detailed description of the method, with applications, elsewhere.

J. E. S. Bradley
J. Marks

Physics Department,
Middlesex Hospital Medical School,
London, W.1.

[1] Mössbauer, R. L., *Z. Naturforsch.*, **14**, 211 (1959).
[2] Aksenov, S. I., Alfimeakov, V. P., Lushchikov, V. I., Ostanovich, Yu. M., Shapiro, F. L., and Yang, U-Huang, *Zh. Eksper. Teoret. Fiz.*, **40**, No. 1, 88 (1961).

Investigation with the Electron Microscope of the Sublimation of Ice Crystals at Low Temperatures

Investigations of ice crystals by means of the electron microscope have been made by several investigators[1–4], but most of them were based on replication techniques. Recently, Fernández-Morán[5] reported the results obtained by low-temperature microscopy; he succeeded in stabilizing ice crystals deposited on a cold substrate by maintaining them at low temperatures during observation in the electron microscope with a microbeam of very low intensity. It was noted by him that the ice crystals were predominantly cubic and hexagonal in shape at −120° C. and at −100° to 90° C., respectively, and actually showed corresponding sharp-ring diffraction patterns.

I have been investigating the freeze-drying process of micro-organisms by the use of an electron microscope in which a specimen-cooling device and a 16-mm. cinematograph were incorporated[6]. When the freeze-drying process of yeast cells was being investigated, I happened to observe the process of sublimation of ice crystals which were deposited on the film mounted on a specimen grid.

The electron microscope used for this experiment was a model 6*A*-type made by the Japan Electron Co. Yeast cell suspension as an experimental material was sprayed on a carbon–collodion film which had been designed to keep the specimen from contamination during irradiation with the electron beam. The droplets sprayed on the film were rapidly frozen to −196° C. by immersing them into liquid nitrogen. The specimen cartridge was quickly transferred into the specimen chamber of the electron microscope and settled in the specimen holder which was precooled to − 140° – − 160° C. by contact with a cooling device refrigerated by liquid nitrogen. Electron beam intensity was controlled to be as low as possible so that the beam might not make artefacts. The electron current density used in this experiment was 5·6 × 10⁻⁴ amp./cm.² which was sufficient for taking a cinematograph at a moving speed of 1·5 frames/sec.

Throughout the specimen field, large circular particles with diameters of 20–50μ were scattered. These proved to be droplets of yeast cell suspensions when observed in a subsequent drying process. In the interspaces numerous small particles could be seen. These particles were of cubic, hexagonal, round or other atypical shape with sizes varying from

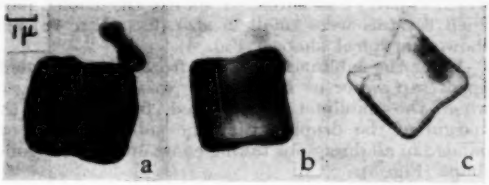


Fig. 1

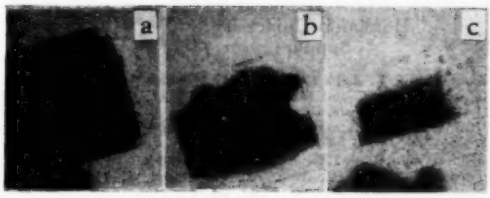


Fig. 2

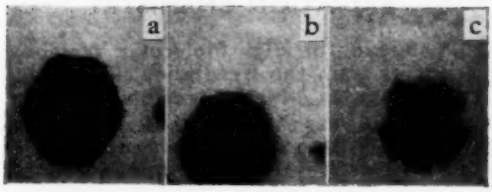


Fig. 3

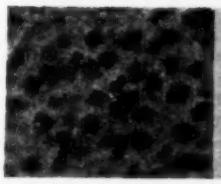


Fig. 4

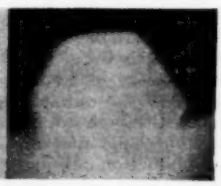


Fig. 5

1 to 5μ; it was quite probable that they were the ice crystals made up of water vapour condensed on the collodion film during transfer from liquid nitrogen to the specimen chamber. Electron diffraction patterns were also obtained corresponding to several crystal types.

After the refrigerant reservoir was raised from the specimen holder, the temperature of the specimen increased gradually. When it reached −90° or −80° C., not exactly measured, the ice crystals began to sublime. As sublimation proceeded, crystals decreased in size and sometimes suddenly disappeared from the field, being probably removed from the collodion film to the vacuum pump. In some cases, ice crystals maintained their contour even after sublimation. This fact was considered to be a kind of replication caused by the electron beam in the microscope during sublimation (Fig. 1,*a*, *b*, *c*). In rare cases it happened that the crystals of cubic and hexagonal types sublimed in such a manner that several 'worm-eaten' spots appeared at the corners of their margins (Figs. 2*a*, *b*, *c* and 3*a*, *b*, *c*). In one case where it was supposed that a temperature gradient may have arisen within the specimen, the water vapour which sublimed from ice crystals at the

warmer part was sublimed on to the cooler part. Such crystals were small in size (less than 1μ) and showed atypical shapes (Fig. 4).

As for the sublimation of the frozen droplets made up of tap water as material, it was once observed, when the sublimation occurred from inside the margin of the droplet, that the sublimed area expanded in all directions taking constantly a hexagonal shape (Fig. 5).

Tokio Nei

Institute of Low Temperature Science,
Hokkaido University,
Sapporo.

[1] Hall, C. E., *J. App. Phys.*, **21**, 61 (1950).
[2] Meryman, H. T., and Kafig, E., *Naval Med. Res. Inst. Proj. Rep. NM000 018.01.09*, **13**, 529 (1955).
[3] Honjo, G., Kitamura, N., Shimaoka, K., and Mihama, K., *J. Phys. Soc. Japan*, **11**, 527 (1956).
[4] Hibi, T., and Yada, K., *J. Electronmicroscopy, Chiba*, **7**, 21 (1959).
[5] Fernández-Morán, H., *Ann. New York Acad. Sci.*, **85**, 689 (1960).
[6] Nei, T., *Biochim. Biophys. Acta*, **49**, 426 (1961).

RADIATION CHEMISTRY

Production of High Molecular Weight Products from the Irradiation of Methane with 4-MeV. Electrons

The effects of ionizing radiations on gaseous methane have been examined previously and the yields of hydrogen and of higher hydrocarbons up to pentane determined to within what appear to be small limits of error[1,2]. Hydrogen is the major product, while the amounts of gaseous hydrocarbons are much less and decrease rapidly with increase in molecular weight. However, there is a 10–30 per cent excess of elemental hydrogen in the products[1–3]. In fact, a large excess of hydrogen seems to be a common feature of the radiation chemistry of gaseous hydrocarbons, though not of liquids[4–6]. It has been known for some time that massive radiation doses lead to the production of liquid products from low-molecular-weight saturated hydrocarbons[7–9], while more recently a more detailed study of liquid product formation from methane and other gaseous hydrocarbons has been reported[10,11]. The nature of the liquid products and their significance in the overall radiolytic process have not been examined.

We have irradiated purified (99·98 per cent) gaseous methane in 'Pyrex' vessels with electrons from a linear accelerator. The dose-rate was about 10^{18} eV. cm.$^{-3}$ min.$^{-1}$ in methane at 1 atm., 15° C. The dosimetry was based on the yield of ethane, $G = 2{\cdot}00$, from methane after short irradiations (< 1 per cent decomposition). Gaseous products were analysed by gas chromatography, liquid products by gas chromatography and mass spectrometry. The liquid was collected by centrifugation. Irradiations giving up to 30 per cent methane decomposition were carried out.

The production of gaseous products was very similar to that found by Lampe[1] except that we were unable to detect unsaturated products irrespective of the dose given. No hydrocarbons above pentanes could be detected in the gas.

Of the liquid product collected, only a small fraction (about 10 per cent) was detectable by gas chromatography at 180° C., and consisted of C_6–C_{12} hydrocarbons, the proportion of C_6 and C_7 being less than that of C_8 and C_9. A further analysis on a much less sensitive temperature programmed gas chromatography apparatus showed that the major portion of the liquid consisted of C_{14} to C_{28} hydrocarbons. A mass spectrometric analysis gave the same overall picture and also showed not only the presence of hydrocarbons with up to 27 carbon atoms per molecule, but also the absence of significant amounts of unsaturated material. Furthermore, even higher hydrocarbons were undoubtedly produced which were not collected by centrifuging the reaction vessel. Irradiation of a methane–argon mixture containing 0·65 electron fraction argon gave essentially the same yield of liquid products as with pure methane. In this case the yield was based on the total energy absorbed in the mixture, using electron fractions to convert from pure methane to the methane–argon mixture. Examination of the gas chromatograms showed, however, that the C_6–C_{12} fraction of the product from the methane–argon mixture was only about half that of the corresponding fraction obtained from pure methane. This shift to higher molecular weight products in argon–methane mixtures may have been due to a gross energy transfer from the argon to methane, resulting in a larger energy deposition per gram of methane in the mixture than in the pure methane. However, irradiation of pure methane to twice the dose given to 50–50 argon–methane mixtures still showed a much larger fraction of low molecular weight liquid products from the pure methane.

The conclusion is drawn that a process occurs both in pure methane and in methane–argon mixtures which results in the formation of high molecular weight products, and that this process is enhanced in the presence of argon (but not of xenon, as shown by other experiments).

Other work on the same systems in this Laboratory has shown some similarity in the behaviour of methane and methane–argon mixtures when irradiated to low doses. For example, the yields of ethane and propane are practically unchanged at argon electron fractions 0–0·9. However, the hydrogen yield, based as usual on the total energy given to the mixtures, is actually greater in the mixtures than in pure methane. It is noteworthy that the additional hydrogen is not scavengable. Also, the *n*-butane/isobutane ratio is significantly increased. In xenon and helium, however, the behaviour is quite different, for example, the *n*-butane/isobutane ratio in methane–xenon mixtures is only about one-third of that observed in mixtures with argon.

The results outlined briefly here indicate that a process occurs in irradiated methane which is enhanced in mixtures with argon, this process giving rise to both increased yields of high molecular weight products and of molecular hydrogen. Since only about half the hydrogen from pure methane is scavengable and the non-scavengable fraction is increased in argon mixtures, it appears likely that a non-radical process is responsible.

It is suggested that the high molecular weight products and non-scavengable hydrogen are produced by ion-molecule reactions[12,13] of the type:

$$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2$$
$$C_2H_5^+ + CH_4 \rightarrow C_3H_7^+ + H_2, \text{ etc.}$$

Melton[14] has recently shown mass spectrometrically that there is a large increase in the CH_3^+/CH_4^+ ratio on addition of argon to methane. The fact that $C_3H_7^+$, or any other higher ion, has not been detected in mass spectrometric studies of methane is not good evidence that this ion is absent or unimportant at the

much higher pressures and lower temperatures used in this work. Indeed, recent work by Rudolph and Melton[15] and by Field[16] has shown that higher molecular weight ions become more important in the mass spectra of acetylene and of ethylene, respectively, when high pressures (up to 350μ in ethylene) are used.

R. W. Hummel

Wantage Research Laboratory,
Atomic Energy Research Establishment,
Wantage,
Berks.

[1] Lampe, F. W., *J. Amer. Chem. Soc.*, **79**, 1055 (1957).
[2] Yang, K., and Manno, P. J., *J. Amer. Chem. Soc.*, **81**, 3507 (1959).
[3] Mains, G. J., and Newton, A. S., *J. Phys. Chem.*, **65**, 212 (1961).
[4] Hardwick, T. J., *J. Phys. Chem.*, **64**, 1623 (1960).
[5] de Vries, A. E., and Allen, A. O., *J. Phys. Chem.*, **63**, 879 (1959).
[6] Freeman, G. R., *J. Chem. Phys.*, **33**, 71 (1960).
[7] Lind, S. C., and Bardwell, D. C., *Science*, **62**, 422 (1925).
[8] Lind, S. C., and Bardwell, D. C., *J. Amer. Chem. Soc.*, **48**, 2335 (1926).
[9] Honig, R. E., and Sheppard, C. W., *J. Phys. Chem.*, **50**, 119 (1946).
[10] Mikhailov, B. M., Tarasova, L. V., and Bogdanov, V. S., *Izd. Akad. Nauk S.S.S.R.*, 218 (1958).
[11] Bogdanov, V. S., *Russ. J. Phys. Chem.*, **34** (5), 496 (1960); *Doklady A.N., S.S.S.R.*, **136** (1), 121 (1961).
[12] Schissler, D. O., and Stevenson, D. P., *J. Chem. Phys.*, **24**, 926 (1956).
[13] Meisels, G. G., Hamill, W. H., and Williams, R. R., *J. Phys. Chem.*, **61**, 1456 (1957).
[14] Melton, C. E., *J. Chem. Phys.*, **33**, 647 (1960).
[15] Rudolph, P. S., and Melton, C. E., *J. Phys. Chem.*, **63**, 916 (1959).
[16] Field, F. H., *J. Amer. Chem. Soc.*, **83**, 1523 (1961).

CHEMISTRY

Valence State of Platinum–Alumina Catalysts

Platinum-on-alumina reforming catalysts are widely used in the petroleum industry. Recent work in this laboratory has shown that catalysts containing soluble platinum more readily convert paraffins to aromatics[1]. Soluble platinum is that which will dissolve in either dilute hydrofluoric acid or acetylacetone. It is believed to exist as a compound involving the alumina support and the chloride present in the catalyst, and is in the plus-four valence state after solution in dilute hydrofluoric acid.

To establish the valence state of soluble platinum in the catalyst, ultra-violet spectrophotometric measurements have been made on catalysts specially prepared on alumina aerogels[2]. In addition, the reflectance of finely ground samples of previously pelleted platinum-on-alumina catalysts has been measured.

Before spectral analyses of the transparent aerogels could be made, the platinum–chloro-alumina complex was converted to the platinum–iodo-alumina complex. The exchange is necessary because the platinum–chloro-alumina complex absorbs in a region of the ultra-violet spectrum (260 mμ) where scattering by the alumina aerogel is too high to allow observation of the peak. Absorption of the platinum-iodo–alumina complex in the near ultra-violet (360 mμ), however, is free of light-scattering. The platinum–iodo-alumina complex was made from an all-soluble platinum–chloro-alumina aerogel catalyst by carefully treating an evacuated aerogel with a solution of potassium iodide in absolute methanol and normal pentane. The exchange of chloride for iodide is straightforward, with no oxidation of platinum in the potassium iodide solution[3]. The absorption spectra for aqueous iodoplatinic acid and the platinum–iodo-alumina aerogel are shown in Fig. 1. Measurements were made with a Cary model 11-*MS* spectrophotometer. A slight shift of the platinum–iodo-alumina peak to a longer wave-length for the aerogel, as compared with aqueous H_2PtI_6, might be due to additional complexing with the alumina. Because divalent platinous iodide does not absorb in this region[3], these spectra indicate platinum, as complex on the catalyst, is present in the plus-four valence state. Iodide exchange of a platinum–chloro-alumina aerogel that was insoluble in hydrofluoric acid showed no ultra-violet absorption, as indicated by the dashed line.

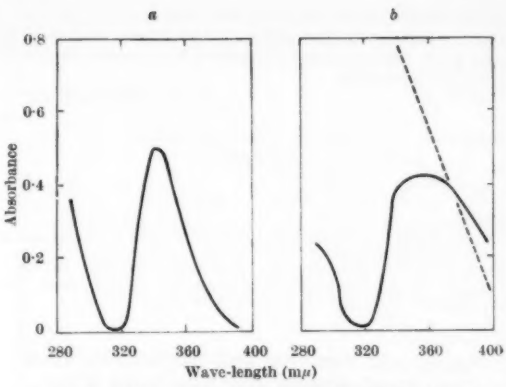


Fig. 1. Ultra-violet spectra. *a*, Aqueous $PtI_6^=$; *b*, catalyst treated with potassium iodide

Reflectance spectra of platinum catalysts are shown in Fig. 2. The results were obtained with a Beckman model *DU* reflectance attachment on samples ground finer than 100 mesh. Magnesium carbonate was the reference standard. An absorption spectrum of aqueous chloroplatinic acid is included for reference. The soluble platinum catalyst shows a small peak slightly shifted from the peak for aqueous $PtCl_6^=$. A sample of alumina alone and the platinum catalyst insoluble in hydrofluoric acid show no peaks.

Platinum in the soluble form is thus in the octahedral plus-four valence state in the catalyst. The

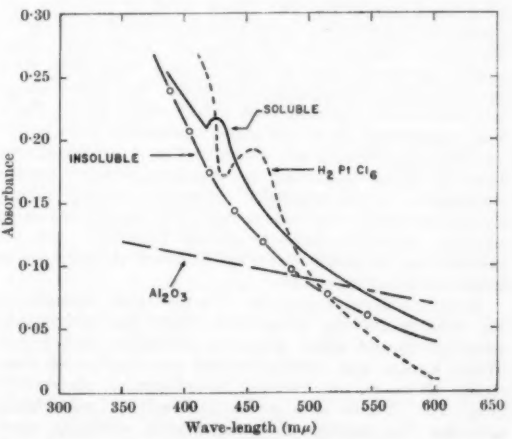


Fig. 2. Reflectance spectra

exact structure of the platinum–chloro-alumina complex is unknown, but attempts to fit six co-ordinate platinum into assumed models of the alumina surface should be fruitful.

R. J. Bertolacini

Research and Development Department,
American Oil Company,
Whiting,
Indiana.

[1] McHenry, K. W., Bertolacini, R. J., Brennan, H. M., Wilson, J. L., and Seeliz, H. S., *Second Intern. Cong. on Catalysis, Paris*, July 4, 1960 (in the press).
[2] Perl, J. B., and Hannan, R. B., *Spectrochim. Acta*, **16**, 237 (1960).
[3] Sandell, E. B., *Colorimetric Determinations of Trace Metals*, 728 (Intersci. Pub., Inc., New York, 1959).

Puzzolanic Activity and Semiconductivity of Fired Kaolin and Kaolinitic Clay

It is known that the reaction of a puzzolan with lime in the presence of water is a chemisorption process[1], and that the puzzolanic activity of a material is enhanced when its crystal structure breaks down, that is, when the material is in a highly disordered state. The enhancement of puzzolanic activity is attributed to the increased reactivity of the materials in the disordered state[2] or its lattice being in strained condition. Similarly, the observed high puzzolanic activity of volcanic ash, a natural puzzolan in the glassy phase, is considered to be the result of the highly strained condition of its lattice[3].

On the other hand, a chemisorption process depends on the electrical characteristics of the reactants[4] and the breakdown of structure of a crystal, say, through the action of heat, may produce crystals which may be semiconductors. Clay minerals, for example, on firing are changed into an intimate mixture of oxides, some of which are semiconductors. It is also possible that the defects produced in the crystal when heated under appropriate conditions may induce semiconducting properties[5]. It is therefore suggested that the effect of disorders or defects present in a natural puzzolan or introduced into a material to improve its puzzolanic activity by heating (as in burnt clays) is to render the material a semiconductor; then the reactivity with lime of a puzzolan may be a semiconductor surface reaction.

Results of work on the puzzolanic activity of kaolin (unfired, fired and fired with zinc oxide) and of a fired kaolinitic clay (fired with or without admixture of zinc oxide) have been published recently[6,7]. Electrical resistivities of some of these materials, kaolin (unfired, fired at 800° C. and fired with 0·01 per cent zinc oxide) and a kaolinitic clay (fired alone and with 0·01 per cent zinc oxide added to it), have been measured to see if these materials were semiconductors. Results are given in Fig. 1, which shows that (*a*) these materials are semiconductors (kaolin undergoes crystallographic changes during firing, hence for kaolin the values represent the resistivities of the products formed during heating) and (*b*) resistivities of these materials increase slightly when heated with zinc oxide.

If the puzzolanic activity of a material depends on its semiconducting properties, then its puzzolanic activity should alter with its electrical resistivity. When kaolin and kaolinitic clay are fired with zinc oxide there is an increase in electrical resistivity (Fig. 1). There is also an increase in puzzolanic activity (in terms of compressive strength and adsorption of lime) of these materials when fired with

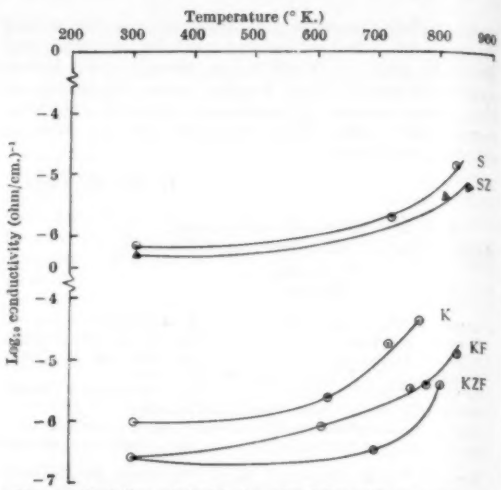


Fig. 1. Variation of electrical conductivity with temperature. K, Kaolin; KF, kaolin fired at 800° C.; KZF, kaolin + 0·01 per cent zinc oxide fired at 800° C.; S, clay fired at 800° C.; SZ, clay fired with 0·01 per cent zinc oxide at 800° C.

zinc oxide[6,7]. Similarly, there is an increase in resistivity of kaolin on firing (Fig. 1) and there is also a corresponding increase in both compressive strength and adsorption of lime of kaolin upon firing[6].

If adsorption of lime by a puzzolan in the presence of water is a chemisorption process on the surface of a semiconductor, there should be a change in the resistivity of the puzzolan after it has adsorbed lime in presence of water. Accordingly, resistance measurements of kaolin and kaolin fired at 800° C. were carried out at room temperature. These materials were then allowed to adsorb lime in the presence of water and their resistances (after having been thoroughly washed and dried) were again measured under similar conditions. It was found that there was a difference in the resistivities of these materials before and after they had adsorbed lime[8]. These resistance measurements were, however, not very reliable.

A natural puzzolan with a glassy structure can be a semiconductor. It is the short-range order (bonding predominantly covalent) which determines semiconductivity rather than the long-range order (structure). The general conditions to be fulfilled by a material in order that it may be a semiconductor have been given by Mooser and Pearson[9]. It will be seen that it is quite possible that many natural puzzolans satisfy these conditions.

If reactivity with lime of a puzzolan is a semiconductor surface reaction, the formation of a compound on the surface of a puzzolan will involve electron transfer and will be guided by the chemical potentials in the adsorbate and in the puzzolan[4] (that is, Fermi-level). Obviously only those semiconducting materials which have suitable Fermi-levels can behave as puzzolans.

It is therefore suggested that: (i) for a material to have puzzolanic properties it must be a semiconductor; glassy-phase puzzolans are probably glassy-phase semiconductors; and (ii) reactivity with lime of a puzzolan then may be considered to be a semiconductor surface reaction.

I thank Shri A. K. Dutta, research officer of the Indian Association for the Cultivation of Science,

Calcutta, for the electrical resistivity measurements. This communication is published by the permission of the Director, Central Building Research Institute, Roorkee.

A. K. Chatterji

Central Building Research Institute,
Roorkee, India.

[1] Krasilnikov, K. G., *Chem. Abstr.*, **52**, 6750 (1958).
[2] Srinivasan, N. R., *J. Sci. Indust. Research* (India), **15**, A, 500 (1956).
[3] Srinivasan, N. R., *Road Res. Paper* No. 1, **26** (1956).
[4] Dowden, D. A., *J. Chem. Soc.*, 242 (1950).
[5] Wells, A. E., *Structural Inorganic Chemistry*, 133 (Oxford, 1950).
[6] Chatterji, A. K., *et al.*, *J. Sci. Indust. Res.* (India), **19**, B, 452 (1960).
[7] Chatterji, A. K., *et al.*, *J. Sci. Indust. Res.* (India), **19**, B, 493 (1960).
[8] Chatterji, A. K., and Roy, S. N. (unpublished data).
[9] Mooser, E., and Pearson, W. B., *Progress in Semiconductors*, **5**, 105 (Heywood Co., Ltd., 1960).

Sedimentation of β-Lactoglobulin (*A*,*B*) under Dissociating Conditions in Acid Solution

Evidence that β-lactoglobulin molecules dissociate into halves in the *p*H region 1·6–4 has been presented by Timasheff, Townend and Weinberger[1–3], and they have shown[3] that there is reasonable agreement between the results obtained by the complementary methods of ultracentrifugation and light-scattering. The overall picture which emerges is of a dumb-bell-shaped[4] lactoglobulin molecule, of axial ratio 2, dissociating reversibly at 25° below *p*H 4 into two spherical sub-units of equal size. According to light-scattering data, and on the assumption of a molecule of weight 36,000, the dissociation constant at *p*H 1·6 is $2{\cdot}5 \times 10^{-4}$ mol./l. and at *p*H 3·5 is $4{\cdot}3 \times 10^{-6}$ mol./l.[3].

We have now attempted to carry a comparison of the two methods a stage further, and to calculate the implications for sedimentation of accepting these values from light-scattering of the dissociation constants.

The main effect of dissociation on sedimentation is seen in a departure of the sedimentation velocity–concentration curve from the normal form. Weak dissociation leads to a high maximum, whereas strong dissociation leads to a lower maximum, or if sufficiently strong, to no maximum and only a change of slope[5]. In Fig. 1 theoretical curves are shown for sedimentation at *p*H 1·6 and 3·5. The sedimentation velocity–concentration curves marked *a* and *b* have been constructed on the basis of the light-scattering dissociation constants, together with the following minimal assumptions: (1) The limiting sedimentation coefficient of the sub-unit (monomer) is 1·89 svedbergs. (2) The whole molecule (dimer) moves relatively faster than the sub-unit by the factor $2^{2/3}$ because of its weight, and 1/1·044 because of its shape[6], that is, $s_2 = 1{\cdot}52\, s_1$. (3) The sedimentation coefficient for each species considered alone obeys the relation $s = s_c\,(1/1 + gc)$, where g is a constant. (4) At *p*H 1·6, the maximum value of the sedimentation coefficient, measured using the boundary median, is 2·34 svedbergs. This fixes the value of g as 0·1 (gm./100 ml.)$^{-1}$.

Whether the curves are based on a weight average[7], or the position of the median of the boundary[5,8], is not very material for this simple monomer–dimer system, as was shown earlier[5].

The experimental points in Fig. 1 are taken from the work of Townend, Weinberger and Timasheff[3], and include one check point not hitherto published

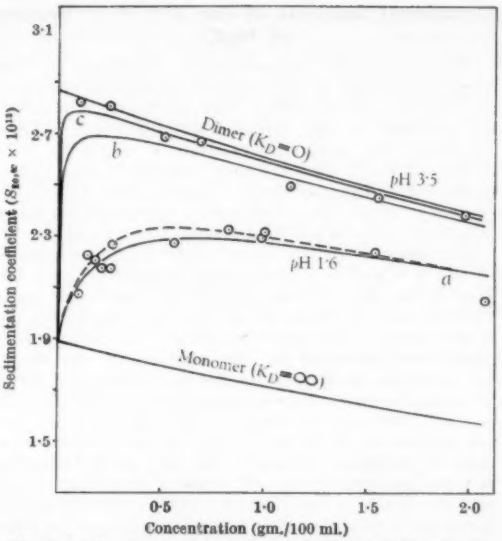


Fig. 1. Sedimentation of lactoglobulin (*A*,*B*) under dissociating conditions. *a*, $K_D = 2{\cdot}5 \times 10^{-4}$ mol./l.; *b*, $K_D = 4{\cdot}3 \times 10^{-6}$ mol./l.; *c*, $K_D = 4{\cdot}3 \times 10^{-7}$ mol./l. ——, Weight average (theory); – – –, median of boundary (theory); ○, experiment (Townend, Weinberger and Timasheff, ref. 3)

(Timasheff and Townend, personal communication). It is seen that they lie fairly near the theoretical curves *a* and *b* when considered as a whole, but that there is an obvious discrepancy at low concentrations at *p*H 3·5. It is difficult to see how a dissociation constant as great as $4{\cdot}3 \times 10^{-6}$ mol./l. can be reconciled with the sedimentation results at the two lower concentrations there. The effect on the position of the theoretical curve of reducing the value of the dissociation constant ten-fold is shown in Fig. 1 (curve *c*). It will be interesting to see whether future work on purified *A* and *B* lactoglobulin eliminates this conflict between the interpretation of light-scattering and ultracentrifuge data.

The value of g is in itself of some interest, since it is about twice that to be expected for a non-reacting protein near its isoelectric point. This may be a reflexion of the fact that the lactoglobulin molecules are sedimenting in a highly charged condition, and that electrostatic repulsion is reducing the number of near neighbours, and hence their contribution to the net velocity of sedimentation[9].

This work was supported in part by a grant from the Agricultural Research Service, U.S. Department of Agriculture.

Lilo M. Gilbert
G. A. Gilbert

Department of Chemistry,
The University,
Birmingham 15.

[1] Townend, R., and Timasheff, S. N., *J. Amer. Chem. Soc.*, **79**, 3613 (1957).
[2] Timasheff, S. N., and Townend, R., *J. Amer. Chem. Soc.*, **83**, 470 (1961).
[3] Townend, R., Weinberger, L., and Timasheff, S. N., *J. Amer. Chem. Soc.*, **82**, 3175 (1960).
[4] Green, D. W., and Aschaffenburg, R., *J. Mol. Biol.*, **1**, 54 (1959).
[5] Gilbert, G. A., *Nature*, **186**, 882 (1960).
[6] Perrin, F., *J. Phys. Radium*, **7**, 1 (1936).
[7] Svedberg, T., and Pedersen, K., *The Ultracentrifuge*, 28 (Oxford University Press, London, 1940).
[8] Gilbert, G. A., *Proc. Roy. Soc.*, A, **250**, 377 (1959).
[9] Gilbert, G. A., *Abst. Intern. Biophys. Cong. Stockholm*, 312 (1961).

Rotational Analysis of the $A^2\Pi$–$X^2\Sigma^+$ System of MgCl

Although the internuclear distances in the diatomic halides of the typical elements of Groups I and III are known, often very precisely, few values are available for the diatomic alkaline earth halides. Following the rotational analysis[1] of the $C^2\Pi$–$X^2\Sigma^+$ system of CaCl, we have now completed an analysis of the 0–0 and part of the 0–1 bands of the $A^2\Pi$–$X^2\Sigma^+$ system of MgCl, from absorption spectrograms taken in a third order of a 6·5-m. grating instrument.

The analysis of the longer-wave-length sub-bands of the 0–0 and 0–1 bands was straightforward, but lines of the shorter wave-length sub-bands are badly blended and overlapped, and the 1–0 bands are too poorly resolved for analysis. However, a satisfactory check was provided by the analysis of the longer wave-length component of the 0–0 band of ^{27}MgCl. The bands observed present no unusual features. As in CaCl, the spin-splitting in the ground-state was too small to be detected, so that the satellite branches were everywhere blended with the main branches. Λ-type doubling in the $^2\Pi$ state is also very small, and the lines in the shorter wave-length sub-bands, presumed to involve $^2\Pi_{\frac{1}{2}}$, are too overlapped to show whether this state is, in fact, inverted, as expected from the likely electron configuration.

Table 1. Summary of Constants (^{25}MgCl)

State	T_e	$\Delta G_{\frac{1}{2}}$	B_e	$10^3\,\alpha$	$10^7\,D$	r_e, Å.
$A^2\Pi_{\frac{1}{2}}$	26,535·89		0·25116	(1·8)	$2·2_5$	$2·171_6$
$A^2\Pi_{\frac{3}{2}}$	26,481·95		0·24914	(1·8)	$2·2_5$	$2·176_4$
$X^2\Sigma^+$	0	462·12	0·24423	$1·5_3$	$2·5_2$	$2·198_8$

A summary of the constants obtained in the present work is given in Table 1. The value for the internuclear distance in the ground-state is $r_e = 2·199$ Å., not significantly different from the value $2·18 \pm 0·02$ Å. obtained for the gaseous dihalide, $MgCl_2$, by electron diffraction[2].

E. Morgan
R. F. Barrow

Physical Chemistry Laboratory,
University of Oxford.

[1] Morgan, E., and Barrow, R. F., *Nature*, **185**, 754 (1960).
[2] Akishin, P. A., *et al.*, see Bastiansen and Lund, *Ann. Rev. Phys. Chem.*, **10**, 43 (1959).

BIOCHEMISTRY

Application of Atomic Absorption Spectroscopy to the Determination of Calcium in Saliva

The importance of salivary calcium concentration of the environment in the solubility of tooth and enamel is readily apparent. As with other biological fluids, numerous methods, ranging from classical oxalate-permanganate titration and emission flame photometry to the more recent complexometric and colorimetric techniques, have been used to estimate the calcium content of saliva. Unfortunately most of these methods have certain disadvantages. The oxalate-permanganate method requires relatively large amounts of saliva, time-consuming centrifugation, washing and titration within a narrow temperature-range. Its accuracy has been questioned because of incomplete precipitation of the oxalate or loss of some calcium oxalate during the washing process[1]. Another devious procedure involves a complexometric analysis of calcium and magnesium in saliva followed by a separate colorimetric determination of magnesium, calcium being obtained as the difference[2]. Several complexon-titration techniques using eriochrome black *T*, murexide or plasmo corinth indicators were tested on saliva. They were found unsatisfactory because the colour shift at the end-point was either too gradual and indistinct or, in the case of murexide, the colour faded too rapidly.

Table 1. Calcium Content (mgm./100 ml.) of Saliva Estimated by Different Methods

Saliva	Type	(*a*) Saliva diluted with water	(*b*) Saliva diluted with ethylenediamine tetra-acetic acid	(*c*) Saliva deproteinized strontium suppressor	(*d*) Saliva deproteinized lanthanum suppressor	Oxalate permanganate method (Clark-Collip)
H.S.	Whole saliva*	—	5·50	5·48	5·68	—
A.T.		—	7·25	7·40	7·50	7·42
J.F.		—	7·15	7·54	7·50	7·52
E.N.‡		3·70	5·08	5·15	—	—
E.N.	Parotid†	2·26	3·35	3·29	—	3·42
A.T.		3·39	5·55	5·55	—	—
H.S.		2·9	3·7	3·2	—	3·6
H.S.		2·46	3·76	3·75	—	3·71
B.G.		2·48	4·23	4·13	—	3·99
E.N.	Submaxillary‡	4·63	7·39	7·37	—	—
H.S.		3·99	6·40	6·40	—	6·95
H.S.		5·02	6·55	6·85	—	—

* Whole saliva was stimulated by paraffin.
† Saliva stimulated by application of citric acid to tongue.
‡ Sample centrifuged to remove particulate matter.

Willis's method[3] for the determination of calcium in blood serum by atomic absorption spectroscopy has been adapted for salivary calcium analysis and found to be accurate and rapid, requiring as little as 0·1 ml. of saliva. The apparatus used in these experiments has been previously described[4]. A Hilger *D* 290 monochromator was found satisfactory in this system.

Certain normal constituents of saliva can interfere in the determination of calcium by the atomic absorption method. Sodium enhances calcium absorption, phosphorus depresses the calcium absorption and the interference of protein appears to be variable, depending on the type of flame used[3]. Saliva can be deproteinized by the standard procedure of adding trichloracetic acid and centrifugation. Large concentrations of $[Sr^{++}]$ or $[La^{+++}]$ can compete with calcium for combination with phosphorus and suppress this interference. Ethylenediamine tetraacetic acid, by complexing calcium, can also overcome phosphorus interference and presumably compete with proteins for calcium.

The results of calcium estimations on saliva are shown in Table 1. The saliva was diluted 1 : 10 by one of the following methods: (*a*) direct dilution with water; (*b*) dilution with water and solution of the disodium salt of ethylenediamine tetraacetic acid, final concentration 10,000 p.p.m. ethylenediamine tetraacetic acid; (*c*) dilution with water, strontium chloride solution and trichloracetic acid, final concentration 2,500 p.p.m. strontium and 4 per cent trichloracetic acid. The sample was centrifuged and measurements made on the supernatant; (*d*) similar to method (*c*) but lanthanum chloride was used as a phosphate 'suppressor', final concentration 10,000 p.p.m. lanthanum.

The saliva thus prepared was measured relative to standard calcium solutions containing respectively: (*a*) 60 p.p.m. sodium (approximate level in 1 : 10 diluted saliva); (*b*) 10,000 p.p.m. ethylenediamine tetraacetic acid; (*c*) 5,500 p.p.m. strontium, 60 p.p.m. sodium and 4 per cent trichloracetic acid; (*d*) 10,000 p.p.m. lanthanum, 60 p.p.m. sodium and 4 per cent trichloracetic acid.

Consistently low calcium values were obtained by the method of direct dilution with water as might be expected due to the presence of interfering phosphorus and protein. The other three methods of preparing solutions for measurement by the atomic absorption technique gave results which were in close agreement and which were in good correlation with calcium values obtained by oxalate–permanganate titration.

Experiments on the recovery of calcium added to saliva in known amounts indicated that, with the exception of direct dilution with water, atomic absorption analysis gave acceptable results. Saliva samples diluted with ethylenediamine tetraacetate acid gave an average recovery of 98·6 per cent and deproteinized saliva containing strontium suppressor gave an average recovery of 99·0 per cent.

From the results in Table 1 it may be deduced that the secretion of the submaxillary salivary glands (6·8 mgm. per cent calcium) contains about 50 per cent more calcium than that of the parotid gland (4·1 mgm. per cent calcium) under conditions of exogenous stimulation. The higher frequency of calculus surrounding lower anterior teeth and their relative immunity to dental caries may be attributable to their proximity to the orifices of the ducts of the submaxillary glands and the higher calcium content of this secretion.

Using Schneyer's data[5] for the proportion of individual gland pair secretions of total stimulated saliva (submaxillary 63 per cent; parotid 34 per cent) and neglecting the contribution of sublingual and other minor glands, the calcium content of whole saliva was calculated as 5·9 mgm. per cent:

$$\left(\frac{6{\cdot}1 \times 63 + 4{\cdot}1 \times 34}{97}\right)$$

Values for calcium content of whole saliva shown in Tables 1 and 2 are in good agreement with this theoretical amount.

The 'debris' and particulate matter which is inevitably present in whole saliva interferes with some of the volumetric and colorimetric methods for determining salivary calcium content. Many investigators have therefore carried out preliminary centrifugation of the whole saliva. Provided that there is no clogging of the capillary tube of the atomizer, the atomic absorption spectrophotometric method can be applied directly to whole saliva diluted in ethylenediamine tetraacetic acid without prior centrifugation. Table 2 shows the results of duplicate calcium analyses on five samples of whole saliva both before treatment and on the supernatant and precipitate following centrifugation. My results indicate an approximately 22 per cent lower calcium value for centrifuged saliva.

Table 2. CALCIUM CONTENT OF WHOLE SALIVA* (MGM./100 ML.) ESTIMATED BY DIRECT DILUTION WITH ETHYLENEDIAMINE TETRAACETIC ACID

Saliva	Untreated saliva	Centrifuged †	
		Supernatant	Precipitate
E.N.	6·52	5·08	1·58
	6·52	5·08	1·68
E.N.	6·00	4·73	2·06
	5·75	4·73	—
E.N.	6·35	4·25	2·78
	6·52	4·08	2·78
H.S.	6·00	5·28	1·25
	6·15	5·08	1·38
B.G.	5·42	4·40	1·25
	5·42	4·40	1·15

* Stimulated by paraffin.
† Centrifuged at 4,000–4,500 r.p.m. for 10 min.

With stimulation by citric acid, very little variation was found in the calcium content of parotid and submaxillary saliva collected as consecutive aliquots during 1·5-min. intervals for 15 min. Over the period studied the glands showed no evidence of depletion of the calcium content of the secretion. There appeared to be no appreciable difference in the calcium content of right and left parotid gland saliva collected simultaneously or in the flow-rate.

I am indebted to Dr. J. B. Willis for his suggestions and Dr. K. W. Knox for his interest and encouragement in these experiments. I also wish to thank Mr. B. Gow for his invaluable assistance in assembling the atomic absorption equipment. This work was supported by the National Health and Medical Research Council of Australia.

ERNEST NEWBRUN*

Institute of Dental Research,
United Dental Hospital,
Sydney.

* Present address: School of Dentistry, University of California, San Francisco Medical Center, San Francisco 22.

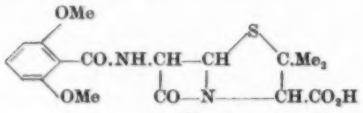
[1] MacIntyre, I., *Biochem. J.*, **67**, 164 (1957).
[2] Ericsson, Y., *J. Dent. Res.*, **34**, 104 (1955).
[3] Willis, J. B., *Spectrochim. Acta*, **16**, 259 (1960).
[4] Box, G. F., and Walsh, A., *Spectrochim. Acta*, **16**, 255 (1960).
[5] Schneyer, L. H., and Levin, L. K., *J. App. Physiol.*, **7**, 609 (1955).

New Penicillins Stable towards Both Acid and Penicillinase

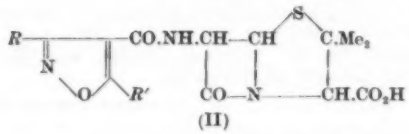
FOLLOWING on the isolation in these Laboratories of 6-aminopenicillanic acid[1], we have used it to synthesize many novel penicillins, with particular emphasis on structures which resist inactivation by staphylococcal penicillinase. One class of penicillins possessing this valuable property has been obtained by acylating 6-aminopenicillanic acid with aromatic or heteroaromatic carboxylic acid chlorides substituted in both *ortho* positions. When the ring structure of the side-chain acid is six-membered, almost any pair of *ortho* substituents confer marked stability towards penicillinase, but when it is five-membered one or both of the substituents must be relatively bulky, for example, phenyl or substituted phenyl. Such findings illustrate the primary importance of steric effects in determining stability towards penicillinase. In this respect the chemical constitution of the side-chain is of much less significance, although it has a profound influence on antibacterial activity and on stability towards acids.

The first such penicillin to be synthesized[2], 2,6-dimethoxyphenylpenicillin (I; methicillin), has proved very effective in staphylococcal infections although, since it is unstable to acid and is poorly absorbed when given by mouth, it has to be administered by injection. We have now prepared a series of 3,5-disubstituted 4-isoxazolylpenicillins[3] (II) many of which, especially those in which one of the *ortho* substituents is a phenyl or substituted phenyl group and the other is a methyl or other lower alkyl group, exhibit high antibacterial activity both *in vitro* and *in vivo*. (We thank Dr. G. N. Rolinson, Mr. D. M. Brown, and their colleagues for carrying out these tests.) Furthermore, they are relatively stable at low *p*H and suitable for oral administration. Thus, 5-methyl-3-phenyl-4-isoxazolylpenicillin (II; R = Ph, R' = Me), 3-methyl-5-phenyl-4-isoxazolylpenicillin (II; R = Me, R' = Ph), and phenoxymethylpenicillin (penicillin V) in 50 per cent aqueous ethanol at *p*H

1·3 and 35° C. all have the same half-life of 160 min. as determined by a previously reported method[4] whereas the half-life of methicillin under the same conditions is between 2 and 3 minutes.



(I)



(II)

A solution of 5-methyl-3-phenylisoxazole-4-carbonyl chloride in acetone was added to a stirred solution of 6-aminopenicillanic acid and sodium bicarbonate in aqueous acetone. After 1 hr. acetone was removed, the mixture was acidified, and 5-methyl-3-phenyl-4-isoxazolylpenicillin was extracted into methyl isobutyl ketone. The clarified extracts were then treated with sodium 2-ethylhexoate in isopropanol, whereupon the hydrated sodium salt of the penicillin crystallized in high yield, m.p. 188° (decomp.), $[\alpha]_D^{20}$ + 201° (c = 1 in water). (Found: C, 52·0; H, 4·9; N, 9·0; S, 7·1; Na, 5·2. $C_{19}H_{18}N_3O_5SNa$, H_2O requires: C, 51·7; H, 4·5; N, 9·5; S, 7·3; Na, 5·2 per cent). A similar reaction with 3-methyl-5-phenyl-isoxazole-4-carbonyl chloride gave the anhydrous sodium salt of 3-methyl-5-phenyl-4-isoxazolylpenicillin, m.p. 239° (decomp.), $[\alpha]_D^{20}$ + 226° (c = 1 in water). (Found: C, 53·7; H, 4·7; N, 9·8; S, 7·6; Na, 5·0. $C_{19}H_{18}N_3O_5SNa$ requires: C, 53·9; H, 4·3; N, 9·9; S, 7·6; Na, 5·4 per cent.)

Details of the preparation of many analogous 3,5-disubstituted 4-isoxazolylpenicillins will be reported elsewhere. Several of the products are at present undergoing clinical evaluation.

F. P. Doyle
A. A. W. Long
J. H. C. Nayler
E. R. Stove

Organic Chemistry Department,
Beecham Research Laboratories, Ltd.,
Brockham Park,
Betchworth, Surrey.

[1] Batchelor, F. R., Doyle, F. P., Nayler, J. H. C., and Rolinson, G. N., *Nature*, **183**, 257 (1959). Doyle, F. P., Nayler, J. H. C., and Rolinson, G. N., Brit. Pat. Spec. 870,396 (1961).
[2] Doyle, F. P., Hardy, K., Nayler, J. H. C., Soulal, M. J., Stove, E. R., and Waddington, H. R. J., *J. Chem. Soc.* (in the press).
[3] Doyle, F. P., and Nayler, J. H. C., U.S. Patent Spec. 2,996,501 (1961).
[4] Doyle, F. P., Nayler, J. H. C., Smith, H., and Stove, E. R., *Nature*, **191**, 1091 (1961).

Cobalt Complexes of Proteins and Polypeptides

We wish to present evidence for the formation of two types of cobalt complexes of proteins and polypeptides in which cobalt is probably bound to the peptide groups. A variety of proteins (for example, gelatin, pepsin, edestin, lysozyme, chymotrypsinogen), as well as tetraglycine and poly-DL-alanine, give the complexes, but most of the work has been carried out with commercial gelatin.

Complex *A* is obtained at high alkalinity as a clear violet solution, $\lambda_{max.}$ 560 mμ. On acidification of the violet complex immediately after formation, the pink colour of cobalt (II) ion is restored. A typical preparation is made as follows. To a mixture of 10 ml. of 10 per cent aqueous gelatin, 1 ml. of 0·35 *M* cobalt (II) sulphate and 4 ml. water, there is added quickly 5 ml. of 4 *M* potassium hydroxide (final hydroxide concentration 1 *M*). The optical density is about 0·3 (0·1-cm. cell) at 560 mμ. Complexes have also been prepared starting with 2 per cent gelatin solutions, the final alkali concentration also being 1 *M*. The more dilute gelatin was used when spectra of complexes of higher cobalt to peptide ratios were measured. The highest molar ratio of cobalt to peptide groups that was found to give the clear violet solution was about 1 : 10. At slightly higher ratios the solutions showed a Tyndall effect probably caused by colloidal cobaltous hydroxide, but were transparent by transmitted light. At still higher cobalt to peptide ratios, the rose-coloured form of cobalt (II) hydroxide was precipitated.

The lowest hydroxide concentration at which a fluid complex of gelatin can be formed is about 0·3 *M*. Below 0·3 *M* alkali a jelly of gelatin combined with the blue form of cobalt (II) hydroxide is produced.

The red-brown complex *B* is formed on ageing of complex *A* in air, the transformation beginning in a few minutes in the case of gelatin, in a few seconds with some other proteins. In an open test-tube the change to red-brown is observed to proceed slowly from the top downwards. A similar change is produced immediately by addition of hydrogen peroxide or hypobromite ion. When a gelatin–cobalt solution was mixed with alkali in vacuum, the violet colour persisted for 22 hr. (although some brown precipitate formed toward the end), at which time air was admitted, resulting in conversion to complex *B* in a few minutes. The absorption maximum of complex *B* is 460–470 mμ. Acidification of complex *B* does not restore the cobalt (II) colour, but gives a light brown solution of $\lambda_{max.}$ 480 mμ.

Apparently *A* is a cobalt (II) complex, while *B* is a cobalt (III) complex. Ligation of cobalt to the peptide groups, with ionization of the peptide hydrogen, is suggested by the high alkalinity required and by the lack of reactivity of poly-L-proline, poly-L-hydroxyproline or poly-sarcosine. The complexes of gelatin with high cobalt contents do not gel at 0°, while a control lacking cobalt, but at the same alkali concentration, does gel. The solutions gel after acidification. By analogy with the biuret-type copper and nickel complexes, which are known to be peptide complexes[1] and also do not gel, we may regard the cobalt complexes as peptide complexes. Tetraglycine binds one cobalt to two tetraglycines; assuming the cobalt to be octahedral it is possible that the ligating groups are the six peptide groups and not the terminal groups. Complex *B* is probably related to the wine-red complexes of cobalt (III) with glycylglycine and glycylglycylglycine which Manyak, Murphy and Martell proposed as having cobalt–peptide (as well as cobalt–amino and cobalt–carboxyl) bonds[2]. The spectra of complexes *A* and *B* prepared from gelatins in which all the amino-groups had been acetylated or 70 per cent of the guanidino groups removed by alkaline hypobromite were the same as those of the complexes of the parent gelatin. (To prevent oxidation of complex *A* a few μgm. of bisulphite were required to destroy traces of positive bromine not removed by dialysis of the hypobromite-treated

gelatin.) In an earlier communication[3] dealing mainly with inhibition of gelation of gelatin by alkaline copper (II), it was reported that a cobalt (II) complex prepared at *p*H 11·5 also prevented gelation. The correct reading is that a *p*H 11·5 nickel (II) complex, $\lambda_{max.}$ 435 mμ, prevented gelation, and that on acidification gelation occurred. The cobalt complex reported earlier was formed at higher alkalinity and was actually the cobalt (III) complex. At that time the cobalt (II) complex was not observed because the very slow adjustment of *p*H permitted oxidation to complex *B* without forming a noticeable amount of complex *A*.

JAKE BELLO
HELENE R. BELLO

Department of Biophysics,
Roswell Park Memorial Institute,
Buffalo, 3.

[1] Martin, R. B., Chamberlin, M., and Edsall, J. T., *J. Amer. Chem. Soc.*, **82**, 495 (1960).
[2] Manyak, A. R., Murphy, C. B., and Martell, A. E., *Arch. Biochem. Biophys.*, **59**, 373 (1955).
[3] Bello, J., and Vinograd, J. R., *Nature*, **181**, 273 (1958).

Formation of 2-Heptanone from Caprylic Acid by Spores of Various Filamentous Fungi

IT was shown recently that washed, non-germinating spores of *Penicillium roqueforti* are capable of producing 2-heptanone from caprylic acid[1]. In examining the mechanism of this reaction[2], other fungal spores were examined for their ability to produce a ketone from the fatty acid. It is significant that spores from almost half the fungi selected converted caprylic acid to heptanone, thus indicating the more general occurrence of a reaction thought to be limited to only a few moulds.

The fungi were grown on tomato juice–agar slants in Roux bottles, and after incubation at 25° C. for 3–10 days (depending on the growth-rate of a particular fungus) the spores were collected by adding water to the bottle and scraping the surface of the mycelium with a flattened metal rod. This mixture was then filtered through several layers of cheesecloth to remove bits of agar and hyphal cells. The spores were removed from suspension by centrifugation and then 'washed' several times in distilled water by centrifugation. The spore concentration was adjusted to 10^9/ml. by using a conventional hæmacytometer. Ketone-forming activity remained constant over a period of several months when the spores were stored in water at 4° C.

To avoid using large amounts of spores, which required more time in culturing and collection, the reaction was conducted in Warburg vessels. The reaction mixture consisted of 1·0 ml. of spore suspension, 1·0 ml. of 0·5 *M* phosphate buffer at *p*H 6·0 or 7·0 and 0·2 ml. of 0·1 *M* sodium caprylate; 0·2 ml. of 20 per cent potassium hydroxide was placed in the centre well to absorb carbon dioxide. Thus, the oxygen consumption of the spores could be followed manometrically and the general direction and rate of the reaction could be observed. Heptanone production was proportional to the consumption of oxygen; if no uptake of oxygen was observed, no heptanone was produced. After 3–5 hr. the Warburg vessels were removed from the bath and the contents of the vessels were steam-distilled directly into 1·0 ml. of a 0·1 per cent solution of 2,4-dinitrophenylhydrazine. The samples, collected in the hydrazine solution, were stored at 4° C. over-night. The following day the hydrazone crystals were washed, recrystallized from hot 95 per cent ethanol and the melting points determined. The hydrazones were also chromatographed at room temperature in a closed glass cylinder with absolute methanol/water (9 : 1 by volume) as the developing solvent. The compounds were spotted on a sheet of Whatman No. 1 filter paper and after 6 hr. development the R_F of the unknown was compared with that of the known hydrazone of 2-heptanone.

It is noteworthy that spores from the majority of the *Aspergillus* sp. (9 out of 11) and *Penicillium* sp. (9 out of 12) and some related moulds including *Paecilomyces varioti* and *Scaputariopsis brevicqulis* rapidly converted caprylate to 2-heptanone. However, none of the spores of Mucorales (*Mucor hiemalis*, *Mucor mucedo*, *Phycomyces blakesleanus*, *Rhizopus stolonifer*, *Thamnidium elegans*, and others) was capable of the reaction.

Spores of *Penicillium roqueforti* and *Aspergillus ochraceus* were found to produce identically the same amount of 2-heptanone. Some of the fungal spores produced more ketone at *p*H 6·0 and others more at *p*H 7·0. Although the reaction with some of the spores may have occurred at a lower or higher *p*H, our only interest in these particular investigations was to determine the range of the reaction among the fungi selected. The pathway for the conversion of fatty acids to ketones by spores of filamentous fungi is being investigated and the results of these experiments will be published shortly.

We are indebted to Prof. W. F. Whittingham and Prof. K. B. Raper for furnishing the cultures for these experiments. This work was supported in part by grant *E*–1201 from the U.S. National Institutes of Health.

R. F. GEHRIG
S. G. KNIGHT

Department of Bacteriology,
University of Wisconsin,
Madison, 6.

[1] Gehrig, R. F., and Knight, S. G., *Nature*, **182**, 1237 (1958).
[2] Haidle, C. W., Gehrig, R. F., and Knight, S. G., *Bacteriol. Proc.*, 157 (1960).

Isolation of a Cystine Derivative from Silk

CONSIDERABLE doubt exists as to whether silk fibroin (*Bombyx mori*) contains a small amount of cystine or whether this amino-acid is entirely absent. Apart from any biological significance, even a very small quantity of cystine, depending on its mode of incorporation, could have a considerable influence on the molecular weight of fibroin.

Schroeder and Kay[1], using ion-exchange chromatography, reported the presence of 0·17 per cent cystine in silk, and later Zuber, Ziegler and Zahn[2] estimated the cystine by an electrophoretic method after oxidation of the fibroin as 0·12 per cent. In recent reviews on silk[3,4], however, no reference is made to its cystine content and it has been asserted that sulphur-containing amino-acids are entirely absent and the claim that silk fibroin contains 0·12 per cent cystine cannot be reconciled with the analytical results so far available[4]. In these laboratories it has been found possible apparently to detect cystine in silk hydrolysates by both paper and ion-exchange chromatography and the Shinohara method (Earland, C., Stell, J. G. P., Lodge, R. S., and Raven, D. J.,

unpublished results). A serious criticism of all this work is that the identification of cystine, or its oxidation product cysteic acid, depends on non-specific colour reactions, and its presence is inferred rather than proved.

It is now shown that to isolate the 2:4-dinitrophenyl derivative of cysteic acid from silk hydrolysates in quantities sufficient for positive identification by analysis is possible. The material isolated can be determined quantitatively, for example, colorimetrically using a Spekker photoelectric absorptiometer, and the minimum cystine content of silk can thus be found. Preliminary work indicates that for *Bombyx mori* this value is about 0·13 per cent. The analytical aspects of this work will be discussed elsewhere, but in agreement with other workers[1,2] it seems very probable that this figure is near the true value. The isolation of the cystine derivative was performed as follows.

Silk fibroin (*Bombyx mori*) was degummed in solution of *p*H 1·5 at 95° C. (ref. 5), the usual alkaline method being avoided to prevent destruction of cystine. The washed and dried fibroin (10 gm.) was refluxed for 8 hr. with hydrochloric acid (150 ml.) and the solution evaporated to dryness *in vacuo* over solid sodium hydroxide. The residue was dissolved in water (200 ml.), neutralized with sodium bicarbonate (20 gm.) and further sodium bicarbonate (40 gm.) was added together with 2:4-dinitrofluorobenzene (25 ml.) in ethanol (300 ml.). The whole was shaken for 12 hr. in an open flask and filtered. The residue was washed with water (100 ml.) and the washings, together with more water (300 ml.), were added to the alcoholic solution. The solution of the dinitrophenyl derivatives was extracted with ether (ethereal solution rejected), acidified with concentrated hydrochloric acid and extracted twice more with ether. The ethereal solution of the dinitrophenyl derivatives was evaporated to dryness, 4 per cent performic acid (50 ml.) added, the solution allowed to stand overnight and again evaporated to dryness. The residue was shaken for 1 hr. with water (50 ml.), filtered and the solid extracted twice more with two further volumes (50 ml. each) of water. The combined aqueous extracts were extracted twice with ether (ethereal extracts rejected) and evaporated to 5 ml.

Electrophoresis of this solution was carried out using a Shandon continuous electrophoresis apparatus (No. 2520) with Whatman 3 *MM* paper using 33 per cent acetic acid as solvent. It was more satisfactory to dispense with the nylon envelopes enclosing the electrodes. Using 300 V. which gave a current of 16 m.amp. the yellow solution of the dinitrophenyl derivatives separated into two sharp bands. Band I moved vertically down the paper with a slight deviation towards the cathode, the solution from this band being rejected. A second band (II) of equal intensity moved strongly towards the anode. The solution from band II was collected and evaporated to dryness. The orange-brown very deliquescent solid was dissolved in acetone, filtered and carbon tetrachloride added to the solution. The whole was evaporated to dryness and kept over phosphorus pentoxide. This material and the authentic dinitrophenyl derivative of cysteic acid migrated identically when subjected to electrophoresis. Yield, 35·4 mgm.

Found (Weiler and Strauss): C, 32·8; H, 3·0; N, 11·8; S, 9·0. $C_9H_9O_9N_3S$ requires: C, 32·3; H, 2·7; N, 12·5; S, 9·5. Synthetic $HO_3SCH_2CH(COOH)NHC_6H_3(NO_2)_2$ (found) C, 32·0; H, 3·0; N, 11·9; S, 9·3.

We wish to thank the Wool Textile Research Council for financial assistance towards this investigation.

C. Earland
D. J. Raven

Department of Textile Industries,
Institute of Technology,
Bradford.

[1] Schroeder, W. A., and Kay, L. M., *J. Amer. Chem. Soc.*, 77, 3908 (1955).
[2] Zuber, H., Ziegler, K., and Zahn, H., *Z. Naturforsch.*, 126, 734 (1957).
[3] Speakman, J. B., *J. Text. Inst.*, 51, P, 392 (1960).
[4] Howitt, F. O., *J. Text. Inst.*, 51, P, 238 (1960).
[5] Burgess, A. F., British Patent 563,745 (1944).

Hydrolysis of α-Sulphoquinovosides by β-Galactosidase

Every photosynthetic tissue so far examined has been found to contain a high concentration of a sulpholipid[1] the structure of which has been elucidated as 6-sulpho-α-D-quinovopyranosyl-(1→1′)-2′,3′-diacyl-D-glycerol[2,3]. A high concentration of the deacylated form of the sulpholipid, *G*-quinSO_3H, occurs in *Chlorella* and *Scenedesmus*[4]. This unique glycoside is cleaved to yield glycerol and 6-sulpho-D-quinovose by a highly purified preparation of β-galactosidase from *Escherichia coli ML* 308 (ref. 5).

Enzymatic reactions were carried out at *p*H 7·0 25° C. with tracer amounts of *G*-quinSO_3H labelled with sulphur-35 with or without synthetic carrier[6]. The hydrolysis followed typical first-order reaction kinetics when the radioactive substrate (approximately 10^{-8} *M*) was used without carrier. Hydrolysis of *G*-quinSO_3H by β-galactosidase was inhibited by the addition of a normal substrate, β-D-galactopyranosyl glycerol (kindly supplied by Prof. H. E. Carter), to the reaction medium, suggesting that the reactions of the enzyme with both types of substrate take place at the same site on the enzyme.

2-Hydroxyethyl or 3-hydroxypropyl sulphoquinovoside exhibited susceptibility to hydrolysis by β-galactosidase. The methyl, ethyl and *n*-propyl glycosides were, however, completely resistant. The β-glycopyranoside and the furanoside isomers as well as α-D-glucopyranosyl-(1→1′)-glycerol were not affected by the enzyme. Sequoyitol and (+)-pinitol (kindly supplied by Dr. Laurens Anderson) did not affect the hydrolysis of the β-galactoside and *G*-quinSO_3H, even though they are related to each other.

These observations indicated that the hydrolyses of the 6-sulphoquinovosides are abnormal but highly specific. β-Galactosidase has been considered strictly specific in its substrate requirement. Only glycosides with the β-D-galactopyranoside structure have been considered susceptible to its hydrolytic action[7]. Since the enzyme attacks certain sulphoquinovosides, as well as β-galactosides, the active site[7] should bind both types of substrate. Although it is difficult to establish a mechanism at this stage, it is interesting that the distances between the oxygen atoms on carbons 1 and 4 are equal in both substrates and that the 2′- or 3′-hydroxyl group of the glycerol and one of the oxygen atoms of the sulphonic acid of *G*-quinSO_3H may occupy relative positions identical with those of the hydroxyl groups of carbons 2 and 3, respectively, of the β-D-galactopyranoside. Since a hydroxyl group of the aglycone was essential for the susceptibility to β-galactosidase action, it must play a part in determining the specificity of the enzyme.

It is interesting that each of the major glycolipids occurring in plants (the sulpholipid and two β-D-galactosyl diglycerides[8,9]) possesses a linkage which may be cleaved by β-galactosidase.

We are indebted to Prof. R. G. Wolfe, of the University of Oregon, for the β-galactosidase preparation, and to Prof. F. J. Reithel, of the same University, for his interest in this work.

This work was supported by a grant from the U.S. National Science Foundation.

ISAO SHIBUYA*
A. A. BENSON†

Department of Agricultural and Biological Chemistry,
The Pennsylvania State University,
University Park, Pa.

* Present address: Institute of Applied Microbiology, University of Tokyo.

† Present address: Laboratory of Nuclear Medicine and Radiation Biology, University of California, Los Angeles, 24, California.

[1] Benson, A. A., Daniel, H., and Wiser, R., *Proc. U.S. Nat. Acad. Sci.*, **45**, 1582 (1959).
[2] Daniel, H., Miyano, M., Mumma, R. O., Yagi, T., Lepage, M., Shibuya, I., and Benson, A. A., *J. Amer. Chem. Soc.*, **83**, 1765 (1961).
[3] Miyano, M., and Benson, A. A., *J. Amer. Chem. Soc.* (in the press).
[4] Shibuya, I., Ph.D. thesis, Pennsylvania State University (1960).
[5] Hu, A. S. L., Wolfe, R. G., and Reithel, F. J., *Arch. Biochem. Biophys.*, **81**, 500 (1959).
[6] Miyano, M., and Benson, A. A., *J. Amer. Chem. Soc.* (in the press).
[7] Wallenfels, K., and Malhotra, O. M., *The Enzymes*, edit. by Boyer, P. D., Lardy, H., and Myrbäck, K., second ed., **4**, 406 (Academic Press, New York, 1960).
[8] Carter, H. E., McCluer, R. H., and Slifer, E. D., *J. Amer. Chem. Soc.*, **78**, 3735 (1956).
[9] Carter, H. E., Hendry, R. A., and Stanacev, N. Z., *J. Lipid Res.*, **2**, 228 (1961).

Interaction between Lysozyme and a Phospholipid of Neoplastic Tissue (Oncolipin)

LYSOZYME is a basic protein, tending to form complexes with fatty acids, aliphatic long-chain alcohols[1], alkyl sulphates[1,2], colic acids[3], and cephalins[4]. Interaction promoted by lysozyme towards Rous sarcoma phosphatides is of particular interest[5].

We have examined the possibility of lysozyme producing insoluble complexes with a phospholipid (oncolipin) isolated in malignant tissues by Aoyama[6].

Tests on interaction have been performed on phosphate 0·067 buffers, using Sörensen's technique.

Oncolipin was isolated from Yoshida's ascite sarcoma in rats. This sarcomatous phospholipid is suspended in saline solution and the above suspension is used for preparing the 0·01 per cent solution of the substance contained in phosphate buffers, at *p*H ranging from 5·9 to 8·2.

Table 1. TURBIDITY RELATED TO HYDROGEN-ION CONCENTRATION, IN SÖRENSEN'S PHOSPHATE BUFFERS

Sample (per cent)	Hydrogen-ion concentration (*p*H) 5·9	6·6	7·1	7·6	8·0	8·2
Lysozyme 0·05	0·004	0·004	0·004	0·004	0·004	0·004
Oncolipin 0·01	0·022	0·020	0·024	0·027	0·029	0·027
Oncolipin 0·01 + Lysozyme 0·001	0·036	0·034	0·032	0·038	0·041	0·038
Oncolipin 0·01 + Lysozyme 0·005	0·051	0·053	0·061	0·065	0·068	0·068

The 0·01 per cent solution of oncolipin in phosphate buffers is slightly turbid. By addition of lysozyme, the turbidity becomes more evident, and proportionally to the added quantity of the enzyme.

It is concluded that lysozyme interacts with oncolipin, at saline hydrogen-ion concentrations, by promoting formation of insoluble complexes. This behaviour of lysozyme is like that of another basic protein, namely salmine, being also an insoluble product interacting with oncolipin, at *p*H 6·8–7·0 (ref. 6).

R. FERRARI
Alexander Fleming Institute of SPA, Milan.

S. MATRACIA
Institute of General Pathology, University, Palermo.

[1] Smith, G. N., and Stocker, C., *Arch. Biochem.*, **21**, 383 (1949).
[2] Klotz, I. M., and Walker, F. M., *Arch. Biochem.*, **18**, 319 (1948).
[3] Caselli, P., *Rass. Med. Sarda*, **5**, 389 (1954).
[4] Brusca, A. E., and Patrono, D., *Giorn. Batt. Virol. Immun.*, **53**, 211 (1960).
[5] Matracia, S., Allegra, D., and Salerno, A., *Comm. Second Intern. Symp. Fleming's Lysozyme, Milan* (1961).
[6] Aoyama, O., *Gann*, **50**, Supp., 270 (1959).

Lysozyme from Human Milk

CONTINUING our research on lysozymes from different origins[1], we were interested in the purification of a human lysozyme. Some preliminary experiments have indicated that human placenta, saliva and spleen contain lysozymes, approximately 4, 10 and 40 mgm./kgm. respectively (quantities expressed in mgm. of hen's egg white lysozyme). In contrast with cow's milk, we have found that human milk contains a lysozyme (40 mgm./l. expressed in mgm. of hen's egg white lysozyme).

We now report the isolation of this new lysozyme which we obtained in a chromatographically pure state.

The purification includes three steps:

(1) Adsorption of the lysozyme on 'Amberlite *CG*–50' buffered with a 0·2 *M* phosphate buffer of *p*H 6·5. 50 ml. of 'Amberlite' are added to 1 litre of milk and the mixture is stirred for 4 hr. The resin is then washed with water and the lysozyme is eluted with a 0·8 *M* phosphate buffer of *p*H 6·5. After dialysis against water at 2° C. and lyophilization, a 'primary material' is obtained: the lysozyme has undergone a 250-fold purification.

(2) Chromatography of the primary material on carboxymethylcellulose. The conditions are indicated in Fig. 1. 200 mgm. of the primary material are chromatographed on a 40 × 1·8 cm. column at 20°. Lysozyme activity was determined by its bacteriolytic action on *Micrococcus lysodeikticus*[2]. After rechromatography of the lysozyme-containing fractions in the same conditions, a symmetrical peak, active against *M. lysodeikticus*, is obtained where the specific activity is constant. In this way, the primary material was again purified 7–8 times.

(3) Desalting of human lysozyme on 'Sephadex *G*–50'. The lysozyme-containing fractions after chromatography on carboxymethylcellulose are pooled, dialysed against water at 2° C. and lyophilized.

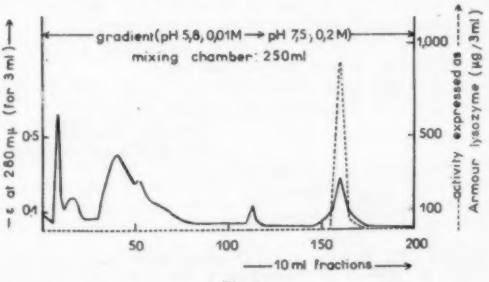


Fig. 1

98 per cent of the salts and only 10–15 per cent of the activity are lost during dialysis. But as only small quantities of lysozyme are involved in the preparation it was necessary to eliminate all the salts without any further loss of activity. This is obtained by filtration on 'Sephadex *G*–50' column 50 × 1·2 cm. (buffer, pyridine 0·3 *M*, HAc 0·1 *M*; *p*H 5·5) (Fig. 2).

Chromatographically pure lysozyme from human milk. After re-chromatography on carboxymethylcellulose, the lysozyme from human milk is obtained with a constant specific activity. Furthermore, no increase of the purity was observed after filtration on 'Sephadex *G*–50'. The fact that the lysozyme obtained in this way is chromatographically pure has been again verified by submitting the enzyme to a ion-exchange (partition) chromatography on 'Amberlite *CG*–50'. The lytic enzyme can be chromatographed on a 20 × 0·9 cm. column buffered with a 0·2 *M* phosphate buffer of *p*H 7·0, without any change of the *p*H, the molarity or the temperature of the buffer during the chromatography (elution after 50 ml.).

Some properties of the lysozyme. The lysozyme from human milk, as all previously examined lysozymes[1], is very stable at acid *p*H: it resists to 100° C. for 3 min. at *p*H 4·5 without any significant loss of activity; it is, however, unstable at alkaline *p*H: at *p*H 7·5 a loss of 30 per cent of the activity occurs after 1 min. at 100° C. If the specific activity is by definition 1 for hen's egg white lysozyme, it is about 2 for human milk lysozyme. The molecular weight is approximately 15,000 (experiments made with calibrated 'Cellophane' membranes). The first results concerning the quantitative amino-acid composition indicate that the new lysozyme is not identical with hen's egg white lysozyme.

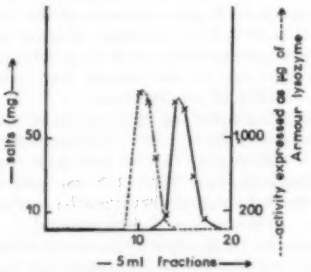


Fig. 2

In any event the new lytic enzyme is a lysozyme as it possesses the principal properties of this group: it is a basic protein, with a molecular weight near 15,000, stable at acid *p*H, unstable at alkaline *p*H, active against suspensions of *M. lysodeikticus*, and liberating by its action on an appropriate substrate compounds which can be detected by reagents for reducing sugars and for *N*-acetylhexosamines.

We are indebted to Mrs. Bertrand (Ecole de Puériculture, Paris) for human milk.

This work was supported by grant *FG–Fr*–106 from the U.S. Department of Agriculture.

Pierre Jollès
Jacqueline Jollès

Laboratory of Biochemistry,
Faculty of Science,
96 Bd. Raspail,
Paris.

[1] Jollès, P., *The Enzymes*, **4**, 431 (Academic Press, New York, 1960).
[2] Jollès, P., *Methods in Enzymology*, **5**, 137 (Academic Press, New York, 1961).

Purification and Concentration of Dye-Protein Conjugates by Gel Filtration

It was shown by Porath and Flodin[1] that filtration through cross-linked dextran gels served as a simple method for the separation of materials of varying molecular size. This procedure has now been found to be of particular value for the purification of antibody-fluorescent dye conjugates for use in the Coon's[2] technique, since unconjugated dye and dye impurities are removed so that non-specific staining of the tissues has thereby been greatly reduced or abolished.

Conjugates of rhodamine[3] and fluorescein isothiocyanate[4] with (*a*) the γ-globulin fraction separated by cold ethanol[5] of rabbit antisera directed against human γ-globulin and β-lipoprotein, (*b*) with whole goat anti-rabbit antiserum and with whole rabbit anti-hen antiserum, were treated as follows: Columns of 'Sephadex *G*–25' (Pharmacia, Ltd., Uppsala) were prepared by shaking 10 gm. of 'Medium Sephadex *G*–25' (100–250 mesh) with 200 ml. of sodium chloride phosphate buffer at *p*H 7·4 for 1 hr. allowing the gel to sediment and decanting the supernatant (including any fine particles). This procedure was repeated twice. The swollen gel was then suspended in a fresh volume of 200 ml. of buffer and slurried into a burette, the lower end of which was plugged with glass wool. Columns varying in length from 10 to 30 cm. × 0·6 cm. diameter were constructed, care being taken to obtain even packing of the gel. The 'void volume' of the column was determined with indian ink.

2–5 ml. aliquots of the protein solutions labelled with dye were applied to the top of the column and allowed to filter through at room temperature. When the conjugate had soaked into the gel, it was eluted with phosphate-saline buffer at *p*H 7·4. Measured volumes of the eluate were collected, the optical density measured at 280 mμ for protein, at 490 mμ (absorption maximum for fluorescein) and 560 mμ (absorption maximum for rhodamine). Aliquots from each eluate were also examined by the Ouchterlony[6] technique and its modification by Gell' against the appropriate antigens for identification and titration of antibody. The eluted conjugate was compared with the original (untreated) material by paper electrophoresis. It was also tested by application to sections cut at −25° C. of mouse- and rat-liver taken from animals injected intravenously with the appropriate antigens and killed immediately, and to human pathological material.

It can be seen from the elution diagram (Fig. 1) that the peak due to protein corresponds to that due to dye in the volume of eluate immediately following the void volume of the column. It was repeatedly observed that a second protein peak (marked by an arrow in Fig. 1) occurred, accompanied by only a slight peak in dye absorption, suggesting partial fractionation of protein which, under the conditions employed for conjugation in these experiments, was less completely labelled with fluorochrome. Further elution of the column gave fractions giving the characteristic absorption of dye alone, unaccompanied by protein.

This sequence could also be observed visually since, during elution, a band of coloured conjugates could be seen to separate from coloured bands of free dye. In the case of rhodamine isothiocyanate, four or five bands differing in colour were seen, suggesting the presence of several impurities in the batch utilized.

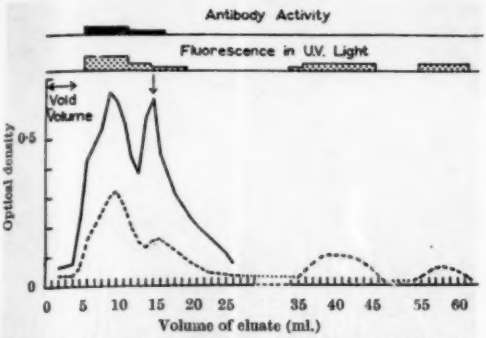


Fig. 1. Elution diagram for an 8-ml. volume of γ-globulin fraction of rabbit anti-human γ-globulin labelled with fluorescein isothiocyanate. The column of 'Sephadex *G*-25', 11 cm. × 0·6 cm. diameter, at room temperature, was eluted with phosphate saline buffer *p*H 7·4. Millilitre eluates were collected. Maximal fluorescence and antibody activity corresponded to eluates of maximal protein and dye absorption. Absorption at 280 mμ, ——; absorption at 490 mμ (dye), – – –

Examination in agar-gel diffusion plates (Fig. 2) shows sharp limitation of antibody activity to the volume of eluate corresponding to the volume of original protein solution and to that giving maximal absorption at 280 mμ (that is, little or no dilution of antibody activity). This was confirmed by the finding that negligible alteration in antibody titre occurred in the eluates. Protein recoveries of up to 95 per cent were obtained in volumes of eluate equivalent to the original volume of the conjugate.

Apart from the use described here, cross-linked dextran gels can also be profitably used for the concentration of fluorescent antibody activity where this is weak, using the method suggested by Flodin, Gelotte and Porath[8] for concentrating solutes of high molecular weight.

Purification of dye-protein conjugates by this method appears to be quicker, simpler and more efficient than other methods[9] at present in use.

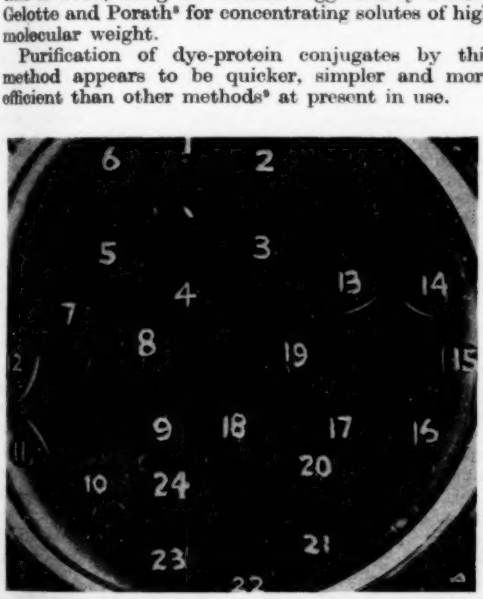


Fig. 2. Agar gel diffusion plate of anti-β-lipoprotein labelled with fluorescein isothiocyanate against β-lipoprotein. After eluting from the column of 'Sephadex *G*-25', samples were taken from each of the ml. eluates and put up against β-lipoprotein. Holes 1-9, representing the void volume of the column, show no precipitation, whereas holes 10-16, representing the volume of protein solution originally put on column, show precipitation. Holes 17-24 show no precipitation

Our thanks are due to Pharmacia Ltd., Uppsala, for free samples of 'Sephadex', and to Dr. C. Ricketts, Medical Research Council Unit, Accident Hospital, Birmingham, for his advice.

W. George
K. W. Walton

Department of Experimental Pathology,
University of Birmingham.

[1] Porath, J., and Flodin, P., *Nature*, **183**, 1657 (1959).
[2] Coons, A. H., Creech, H. J., Jones, R. N., and Berliner, E., *J. Immunol.*, **45**, 159 (1942).
[3] Riggs, J. L., Seiward, J. R., Burckhalter, J. H., and Downs, C. M., *Amer. J. Path.*, **34**, 1081 (1958).
[4] Marshall, J. D., Eveland, W. C., and Smith, C. W., *Proc. Soc. Exp. Biol.* (N.Y.), **98**, 898 (1958).
[5] Lever, W. F., Gurd, F. R. N., Uroma, E., Brown, R. K., Barnes, B. A., Schmid, K., and Schultz, E., *J. Clin. Invest.*, **30**, 99 (1951).
[6] Ouchterlony, O., *Acta Path. Microbiol. Scand.*, **32**, 231 (1953).
[7] Gell, P. G. H., *J. Clin. Path.*, **10**, 67 (1957).
[8] Flodin, P., Gelotte, B., and Porath, J., *Nature*, **188**, 493 (1960).
[9] Creech, H. J., and Jones, R. N., *J. Amer. Chem. Soc.*, **63**, 1661 (1941); **63**, 1670 (1941). Dineen, J. K., and Ada, G. L., *Nature*, **180**, 1284 (1957).

PHYSIOLOGY

Sodium Influx and Excitation in Uterine Smooth Muscle

The applicability of the ionic hypothesis of excitation, more specifically the transient increase of sodium permeability during an action potential[1], has not been extended to smooth muscles in general. This is due largely to serious technical difficulties in impaling the small cells of smooth muscles, as shown by the comparatively low and widely variable values of membrane potentials for smooth muscles. Direct tests of the effect of sodium deprivation on the action potential are therefore rather difficult to make. Additional confusion has been caused by claims that action potentials persist in media containing very little sodium[2] or no sodium at all[3].

An alternative approach to test the applicability of the ionic hypothesis to smooth muscle is to determine the sodium influx associated with each impulse to see whether the charge carried by the sodium ions moving across the excitable membrane can account quantitatively for the displacement of the membrane potential occurring during an action potential. This approach has been used in paired comparisons on isolated myometrial strips from œstrogen-dominated uteri of rabbits. The strips were incubated in a sodium-22 containing Krebs's bicarbonate solution. One of the pair was left unstimulated, and at the incubating temperature of 24° C. spontaneous activity was infrequent. The other of the pair was stimulated in end-to-end fashion by 4-sec. long, 60-c./s. a.c. stimuli, once every minute for about 2 hr. When the relative amounts of sodium-22 eluted from the muscles were plotted against time, the resulting curves could be fitted by exponential equations with two terms. From the intercept values and the rate constants of both terms, the total intracellular sodium-22 at the beginning of the elution was calculated according to the method discussed by Huxley[4].

In paired comparisons of muscle strips allowed to recover for 3 hr. before being loaded with sodium-22 the extra uptake of sodium due to applied stimulation was 16·3 pmoles/cm.²/4 sec. stimulus (mean of 6 pairs, with total stimuli of 120, *S.E.* ± 3·06). Since the stimuli were applied once every minute, allowance must be made for the sodium that entered the cell but was then extruded between stimuli. This correc-

tion, based on the efflux of sodium, increases the sodium uptake to 33 pmoles/cm.²/stimulus. By direct recording, each 4-sec. stimulus was found to elicit, on the average, 4–5 repetitively produced spikes. Thus, the sodium uptake per spike is 7–8 pmoles/cm.². Using the highest value for the spike in uterine smooth muscle of 73 mV.[5] it can be readily shown that movement of this amount of sodium is capable of discharging a membrane capacity of 9–11 μF./cm.², a value which closely resembles that of other vertebrate excitable membranes. On the other hand, if the membrane capacity of the rabbit myometrial cell is assumed to be the same as that of frog sartorius muscle fibre or that of sheep Purkinje fibre[6], the observed and estimated amount of sodium moved could cause a change of membrane potential of about 70 mV. Thus, even without correcting for diffusion delay, which is appreciable in this tissue, the results strongly suggest that the ionic hypothesis of excitation can be applied to the rabbit myometrium.

The half-time of extracellular elution of sodium-22 is 10·3 ± 0·71 min. (mean ± *S.E.* of 22 preparations). From this and the thickness of the preparations the diffusion constant of sodium was calculated to be $0{\cdot}202 \times 10^{-6}$ to $1{\cdot}16 \times 10^{-6}$ cm.²/sec. At the maximum, this is one-fifth the value in frog sartorius muscle[7]. The slow extracellular diffusion together with a higher intracellular sodium concentration[8] could conceivably allow local accumulations of sodium even when the entire preparation is immersed in sodium-deficient media. Probably such an explanation can account for the observation that action potentials in smooth muscles seem to be independent of the external sodium concentration.

This work was supported in part by a grant (*A*–1713) from the National Institutes of Health, U.S. Department of Health, Education and Welfare. Support for one of us (D. Z.) was derived from funds allotted by the U.S. Public Health Service and the National Science Foundation.

C. Y. Kao
D. Zakim

Department of Pharmacology,
State University of New York,
Downstate Medical Center,
Brooklyn, New York.

F. Bronner

Hospital for Special Surgery,
New York, New York.

[1] Hodgkin, A. L., *Proc. Roy. Soc.*, B, **148**, 1 (1958).
[2] Holman, M., *J. Physiol.*, **136**, 569 (1957).
[3] Daniel, E. E., and Singh, H., *Canad. J. Biochem. Physiol.* **36**, 959 (1958).
[4] Huxley, A. F., in *Mineral Metabolism*, **1A**, 163 (Academic Press, New York, 1960).
[5] Marshall, J. M., *Amer. J. Physiol.*, **197**, 935 (1959).
[6] Weidmann, S., *Electrophysiologie der Herzmuskelfaser* (Verlag Hans Huber, Berne and Stuttgart, 1956).
[7] Keynes, R. D., *Proc. Roy. Soc.*, B, **142**, 359 (1954).
[8] Kao, C. Y., *Amer. J. Physiol.*, **201**, 714 (1961).

Effect of Some Smooth Muscle Stimulants on the Motor Response of the Isolated Guinea Pig Vas Deferens to Hypogastric Nerve Stimulation

Recently potentiation of the response of the cat nictitating membrane to sympathetic nerve stimulation has been shown to occur with serotonin[1] and substance *P* (ref. 2). Burn and Rand[3] have found that circulating noradrenaline increases the responses of organs to sympathetic stimulation.

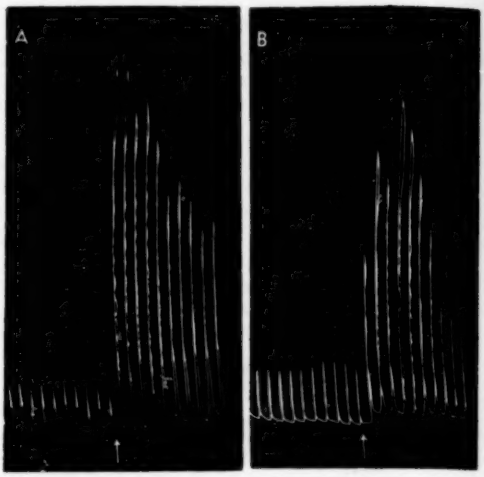


Fig. 1. Contractions of the isolated guinea pig vas deferens in response to hypogastric nerve stimulation (10 V., 2 msec., 5 pulses/sec.) for 5 sec. at 2-min. intervals; bath volume 50 ml. *A*, At arrow, addition of 0·01 μgm. acetylcholine; *B*, at arrow, addition of 5 μgm. adrenaline

Some results are presented here showing that other smooth-muscle stimulants can modify the motor response to nerve stimulation of sympathetically innervated smooth muscular organs. The vas deferens was chosen because this organ is stimulated by most of the smooth muscular stimulants occurring in the body.

The vas deferens with the hypogastric nerve was prepared according to the method described by Huković[4]. The organ was placed in a 50-ml. bath containing Tyrode solution at 37° C. and aerated with oxygen. The contractions were recorded with a frontal writing lever on a smoked drum. The hypogastric nerve was placed on stimulating electrodes and immersed in liquid paraffin. The nerve was stimulated every 1 or 2 min. for 5–10 sec. with a stimulation of supramaximal strength (8–15 V.) and a duration of 2 msec., the frequency being in the range 5–20 per sec. The responses remained for at least 5–6 hr.

Contractions of the isolated guinea pig vas deferens could be elicited by addition to the bath fluid of adrenaline, noradrenaline, acetylcholine, histamine and serotonin although the concentrations required were rather high. Concentrations of at least 5–10 μgm./ml. of adrenaline and noradrenaline had to be used to obtain a moderate contraction. The effective dose of acetylcholine was lower (1–10 μgm./ml.) and those of histamine and serotonin were higher (10–80 μgm./ml.). In general these substances could be ranked in the following order: acetylcholine > adrenaline > noradrenaline > histamine > serotonin.

It was observed, however, that doses of smooth muscular stimulants, which were too small to induce contractions of the isolated vas deferens, caused a conspicuous potentiation of the motor response of the organ to nerve stimulation. Doses ten to a thousand times smaller than those required for eliciting a moderate contraction of the organ increased the response to nerve stimulation two or three times or even more without any gross changes in the basal tone of the ductus. The ability for potentiation followed the same order as that for stimulation. The

potentiation was of long duration, often lasting for 30 min. or more. In this respect there was a difference between the catecholamines and the other smooth muscle stimulants since the effects of adrenaline and noradrenaline rarely exceeded 15 min. and were often shorter. A few experiments were conducted with substance *P*, which was also found to potentiate the response to hypogastric stimulation when doses of 0·2–0·6 unit/ml. were added to the bath.

In order to examine the influence of specific inhibitors on the potentiation described, some experiments were performed with atropine. Atropine in doses of 0·1–1 μgm./ml. abolished the effect of acetylcholine but not those of the catecholamines and histamine.

I wish to thank Prof. U. S. von Euler for help and valuable advice.

This investigation was supported by a grant to Prof. U. S. von Euler from the Thérèse and Johan Andersson Memorial Fund.

NILS O. SJÖSTRAND

Department of Physiology I,
Karolinska Institutet,
Stockholm.

[1] Bacq, Z. M., and Renson, J., *Arch. Intern. Pharmacodyn. Therap.*, **130**, 464 (1961).
[2] Beleslin, D., Radmanović, B., and Varagić, V., *Brit. J. Pharmacol.*, **15**, 10 (1960).
[3] Burn, J. H., and Rand, M. J., *J. Physiol.*, **150**, 295 (1960).
[4] Huković, S., *Brit. J. Pharmacol.*, **16**, 188 (1961).

Non-steroid Inhibitors of Pituitary Gonadotrophic Function

WE have obtained striking changes in the reproductive system of animals of certain species treated with some dithiocarbamoylhydrazines (U.K. patent 878,177). We were prompted to make our experiments by an observation of our colleague, Dr. J. K. Walley.

Among the most active of the compounds we examined is 1-α-methylallylthiocarbamoyl-2-methylthiocarbamoylhydrazine (compound 33,828) :

$$CH_2{=}CH.CH(CH_3).NH.CS.NH.NH.CS.NH.CH_3$$

Administration of this substance to rats of either sex results in selective and reversible inhibition of pituitary gonadotrophic function. We have obtained evidence of similar action in dogs and monkeys, but not in mice, guinea pigs, rabbits or horses.

This action is manifest in various ways. Thus, when female rats with regular œstrus cycles are given daily oral doses of the compound at a level of 20 mgm. or more per kgm., cyclic activity, as judged by vaginal smears, ceases within two or three days. Smears of the dicestrus type persist for as long as dosing is continued: when it is stopped the normal vaginal cycle is resumed within three or four days, even after several months dosing. The ovaries lose weight progressively during administration of the compound and, in histological sections, show a decrease in the number of maturing follicles as compared with normally cycling control animals.

In male rats, daily dosing with the compound at similar levels results in progressive atrophy of the accessory sex glands and a slower loss in weight of the testes. The latter change is associated with arrest of spermatogenesis at the stage of early spermatocytes. The interstitial cells seem to be unaffected. Table 1 shows the percentage reduction in the relative weight of the seminal vesicles, ventral prostate and testes of rats after 10 and 24 daily doses of the compound at various levels, as compared with the relative weight of these organs in control animals of the same age and starting weight. Mean figures are given for groups each of six rats having a body-weight of about 110 gm. at the start of experiment. The treated animals were killed on the day after that on which the last dose was given.

Table 1. PERCENTAGE REDUCTION IN THE RELATIVE WEIGHT OF THE GONADS AND ACCESSORY ORGANS OF MALE RATS (INITIAL BODY-WEIGHT, *c.* 110 GM.) GIVEN DAILY ORAL DOSES OF COMPOUND 33,828

Daily dose of compound (mgm./kgm., p.o.)	Percentage reduction in relative weight After 10 daily doses			After 24 daily doses		
	Seminal vesicles	Ventral prostate	Testes	Seminal vesicles	Ventral prostate	Testes
16	44	21	1	71	61	30
40	66	29	11	88	80	51
100	67	39	6	88	83	55
250	69	49	18	88	85	74

The adrenals and thyroid of rats treated with the compound show some increase in relative weight but no histological abnormality. In the experiment cited the increase was greatest in the animals given 250 mgm. per kgm. for 24 days, and then amounted to 37 per cent for the adrenals and 17 per cent for the thyroid.

The effect of the compound on pregnancy in the rat depends on the level and duration of dosage and the stage at which it is administered. If it is given in sufficient dose (*c.* 100 mgm. per kgm. orally) on any three consecutive days of pregnancy, no litters are obtained. This is probably due, if treatment is given in the early stages of pregnancy, to inhibition of implantation, and if in the later stages, to fœtal death and resorption. However, in animals given doses insufficient to interrupt pregnancy, the surviving offspring have appeared normal. Moreover, the termination of pregnancy in rats by administration of the compound leaves their subsequent fertility unimpaired.

Although the effects of the compound on the reproductive system of rats are apparent with daily doses as low as 16 mgm. per kgm., daily doses of 350 mgm. per kgm. are tolerated for up to six months without serious effects on well-being or on the morphology of other internal organs. Dosage at levels of 16 mgm. per kgm. a day and upward does, however, reduce the rate of gain in body-weight and causes, in some rats, patchy loss of hair. The acute median lethal dose for rats is of the order of 1 gm. per kgm. (single oral dose), at which level perforation of the head of the duodenum is often the cause of death.

The compound does not affect the increase in weight of the uterus of spayed female rats given œstradiol, nor that of the seminal vesicles or prostate of castrate males given testosterone. Atrophic changes produced by its administration in the gonads and accessory organs of sexually mature rats can be prevented by the simultaneous administration of human chorionic gonadotrophin ('Pregnyl'), but the compound does not counteract, in immature animals of either sex, the effects of human chorionic gonadotrophin, pregnant mare serum gonadotrophin ('Gestyl') or human menopausal gonadotrophin. These observations suggest that the action of the compound is central rather than peripheral and this is further supported by observations on the pituitary of treated animals. Although administration of the compound does not significantly affect the cytology of the pituitary in intact rats, it

largely prevents the appearance of castration cells in castrate males, when it is continued from the time of operation until the animals are killed one month later. We therefore conclude, provisionally, that the compound either directly interferes with the production of gonadotrophins by the pituitary, or else affects the presumed hypothalamic control of that function.

Atrophic changes have been detected in the testes and accessory sex organs of dogs given the compound, and in female rhesus monkeys inhibition of menstruation results from its daily administration. We have been unable to obtain any clear evidence of endocrine effects from the compound in mice, guinea pigs, rabbits or horses.

G. E. Paget
A. L. Walpole
D. N. Richardson

Pharmaceuticals Division,
Imperial Chemical Industries Limited,
Alderley Park, Macclesfield,
Cheshire.

Permeability of the Synovial Membrane to Glycoproteins

Electrophoretic investigations have indicated differences in glycoprotein pattern between serum and synovial fluid[1–3]. The most marked difference is that the relative amount of α_2-glycoproteins is considerably less in synovial fluid than in serum. It was recently demonstrated[4] that the low α_2-content of rheumatoid arthritic synovial fluid is mainly due to the low concentration of haptoglobin. The synovial fluid–serum ratio for this glycoprotein was significantly lower than for albumin or γ-globulin. The concentration of the small-molecular glycoprotein orosomucoid was, on the other hand, that expected from the total protein content, indicating a varied permeability of the blood-synovial fluid barrier to glycoproteins.

Therefore, it appeared of interest to extend the investigations to the glycoprotein permeability of the synovial membrane under normal conditions as well as in other cases with joint effusion. Since it has recently been demonstrated[5] that certain coagulation factors appear to be absent from normal bovine synovial fluid, determinations of the coagulation factors prothrombin and proconvertin, which are known to be glycoproteins, were also included in the present work.

Using previously described methods, synovial fluid-serum ratios for haptoglobin[6], orosomucoid[7], prothrombin and proconvertin[8], and certain electrophoretic protein fractions[3] were determined in cases without evidence of joint disease as well as in cases of osteoarthritis and rheumatoid arthritis. The effect of intra-articular administration of corticosteroids was also studied.

The results are summarized in Table 1. The synovial fluid–serum ratios for haptoglobin and prothrombin–proconvertin are seen to be extremely low in the cases without joint involvement (group I *a*) but increase moderately with increasing degree of synovial inflammation. Following intra-articular administration of hydrocortisone in rheumatoid arthritic cases the ratios for haptoglobin and prothrombin–proconvertin decrease markedly. The ratio for orosomucoid, on the other hand, is seen to be closely similar to that for albumin in the various groups.

The cases of group I *b* (Table 1) deserve special mention. In these cases the synovial fluid was of normal amount and appearance and there was no evidence of joint disease. However, in contrast to the cases collected in group I *a*, the patients of group I *b* presented abnormal serum protein patterns, markedly increased serum-level of haptoglobin and orosomucoid, as well as elevated erythrocyte sedimentation-rate. The changes in the synovial fluid–serum ratios for some of the glycoproteins observed in this group were of a degree found in mild synovitis and may possibly reflect a general change in connective tissue permeability to glycoproteins in infectious conditions.

The results indicate that under normal conditions the synovial membrane is very slightly permeable to certain glycoproteins. This semipermeability is obviously not related to the molecular size of the glycoprotein. Already slight synovial inflammation causes marked changes in glycoprotein permeability, which therefore should be a sensitive index of the degree of inflammation. The mechanism of the selective permeability remains unclarified.

Table 1. Synovial Fluid – Serum Ratios of Various Protein Fractions in Cases with and without Joint Disease

Group	No. of cases	Vol. of synovial fluid (ml.)*	Synovial fluid – serum ratios						
			Haptoglobin	Glycoproteins: Orosomucoid	Glycoproteins: Prothrombin-proconvertin	Electrophoretic fractions: Albumin	α_1	α_2	β	γ
I Cases without joint involvement										
(*a*) Without evidence of inflammatory disease	5	0·2–0·3	0·03	0·39	0·10	0·43	0·23	0·13	0·23	0·23
(*b*) With evidence of inflammatory disease (pyelonephritis)	2	0·2–0·3	0·18	0·72	0·27	0·65	0·53	0·35	0·36	0·48
II Osteoarthritis	6	0·5–10	0·24	0·64	0·18	0·46	0·45	0·21	0·32	0·33
III Rheumatoid arthritis										
Untreated	5	10–50	0·38	0·52	0·36	0·57	0·60	0·39	0·51	0·62
After hydrocortisone†	5	2–8	0·18	0·50	0·16	0·58	0·49	0·28	0·42	0·63

* As judged from the amount obtained on puncture.
† Puncture 4 days after intra-articular administration of 50 mgm. hydrocortisone acetate.

Lars Sundblad
Eric Jonsson
Erik Nettelbladt

Central Clinical Laboratory and
Medical Department III,
Södersjukhuset (South Hospital),
Stockholm.

1 Sandson, J., and Hamerman, D., *Proc. Soc. Exp. Med.*, **98**, 564 (1958).
2 Decker, B., McKenzie, B. F., McGuckin, W. F., and Slocumb, Ch. H., *Arthritis Rheumat.*, **2**, 162 (1959).
3 Nettelbladt, E., and Sundblad, L., *Arthritis Rheumat.*, **2**, 144 (1959).
4 Nettelbladt, E., and Sundblad, L., *Arthritis Rheumat.*, **4**, 161 (1961).
5 Cho, M. H., and Neuhaus, O. W., *Tromb. et Diath. Haemorrhag.*, **5**, 108 (1960).
6 Sundblad, L., and Wallin-Nilsson, M., *Opuscula Med.*, **6**, 271 (1960).
7 Sundblad, L., and Wallin-Nilsson, M., *Scand. J. Clin. Lab. Invest.* (in the press).
8 Owren, P., and Aas, K., *Scand. J. Clin. Lab. Invest.*, **3**, 201 (1951).

Dietary Citrate and Hypomagnesæmia in the Ruminant

THE sudden onset of clinical hypomagnesæmia in the lactating cow most commonly occurs soon after the change from stall feeding to grazing fresh spring grass, and in the absence of any marked reduction in intake of magnesium. This condition is probably brought about by reduced absorption of magnesium; but the reasons for this are not fully understood. Marked reductions in output of urinary magnesium on changing the diet from hay or grass nuts to fresh grass have been described for sheep[1] in spite of increased intakes of magnesium; this probably indicates reduced absorption. Care[2] recently calculated that a 50 per cent reduction in absorption could lead to the development of hypomagnesæmia within 7 days in sheep.

Mineral balance of herbage[3], low sodium intakes[4], and increased ruminal ammonia production[5] from the appreciable amounts of soluble nitrogenous constituents of spring herbage have all been suggested as precipitating factors, but none of these has been consistently implicated in clinical cases of the disease. In the course of an investigation into the effects of adding organic acids and their salts to the diet of ruminants, we have obtained evidence that dietary citrate levels may be related to the occurrence of hypomagnesæmia.

Eighteen 4–6 month old Ayrshire heifer calves were housed in individual pens and fed 3·5 lb. of a commercial concentrate, 0·5 lb. crushed barley and hay *ad lib.* to a maximum of 4 lb. daily. The calves were paired and allocated at random to treatments providing (*a*) 30 gm. sodium chloride or (*b*) 50 gm. granular sodium citrate in individual daily doses mixed with the barley. Each supplement provided 11·8 gm. sodium per day.

Intakes of food and gains in live weight were similar for both treatments over an 8-week experimental period. In the eighth week, blood samples for the determination of serum calcium, magnesium and inorganic phosphorus were taken on two occasions with the results shown in Table 1. Those calves which had received sodium citrate showed significantly lower levels of serum magnesium and appreciably lower levels of serum inorganic phosphorus than the calves which had received sodium chloride.

Table 1. EFFECTS OF SODIUM CITRATE UPON SERUM CALCIUM, PHOSPHORUS AND MAGNESIUM (MGM./100 ML. SERUM: MEAN OF TWO OBSERVATIONS PER CALF)

Treatment	Magnesium	Calcium	Phosphorus
(*a*) Sodium chloride	2·11	9·47	9·18
(*b*) Sodium citrate	1·86	9·73	8·43
Significant difference ($P = 0·05$)	0·18	0·52	0·79

The results suggest that the ingestion of small quantities of sodium citrate reduce levels in serum of magnesium and possibly inorganic phosphorus. Since skeletal magnesium is more readily available in the young calf than in the adult cow[6] any factor which interferes with magnesium absorption would be expected to take longer to reduce calf serum magnesium significantly.

The daily intake of citric acid (as sodium citrate) amounted to 1·0 per cent of the dietary dry matter; levels of 0·4–0·7 per cent were reported for fresh perennial ryegrass by Hirst and Ramstad[7]. The occurrence of hypomagnesæmia might thus be associated with increased intakes of citrate from herbage. Further experiments are in progress to extend these observations to the lactating cow and to determine more precisely the effect of citrate intake on magnesium metabolism in the ruminant.

A. W. A. BURT
D. C. THOMAS

Unilever Research Laboratory,
Colworth House,
Sharnbrook,
Bedford.

[1] Field, A. C., *Brit. J. Nutr.*, **15**, 287 (1961).
[2] Care, A. D., *Res. Vet. Sci.*, **1**, 338 (1960).
[3] Brouwer, E., *Brit. Vet. J.*, **108**, 123 (1952).
[4] Ross, D. B., *Nature*, **189**, 840 (1961).
[5] Head, M. J., and Rook, J. A. F., *Nature*, **176**, 262 (1955).
[6] Blaxter, K. L., and McGill, R. F., *Vet. Revs. and Annot.*, **2**, 35 (1956).
[7] Hirst, E. L., and Ramstad, S., *J. Sci. Food Agric.*, **8**, 727 (1957).

Difference in Metabolism of Labelled Thyroxine between Thyrotropic and Adrenotropic Mouse Pituitary Tumours

LITTLE is known about the mechanisms within the pituitary which are involved in the control of thyrotropin secretion exerted by circulating thyroid hormone. In the work reported here mouse pituitary tumours were used to examine this problem since each of these several tumour types reflects quite closely the behaviour of its prototype cell in the normal pituitary. The results suggest there may be a special thyrotropin-secreting cell with an unusual pattern of metabolism for thyroxine. Thus, mouse pituitary thyrotropic tumours were found to contain large amounts of labelled 3,5,3′-triiodothyronine (T_3*) 4 hr. after injection intraperitoneally of labelled thyroxine (T_4*), whereas mouse pituitary adrenotropic tumours and other tissues contained none.

Three strains of thyrotropic tumours in LAF_1 mice, originally made available by Furth[1], were used. Several transplants of an adrenotropic tumour in LAF_1 mice also were furnished by Dr. Furth. A spontaneous fibrosarcoma in LAF_1 mice; kidney, muscle and pituitary from thyroidectomized LAF_1 mice; and a carcinoma of the breast in Swiss mice from Dr. S. Graff were examined as control tissues.

With mouse pituitary tumours, valid conclusions generally may be drawn from groups of 4 mice each (Furth, J., personal communication). Nevertheless, all experiments were repeated for confirmation. Groups of 5 mice each were used for the thyrotropic tumours, and groups of 3 mice each with the other tumours due to a limited number of mice available.

Since two of the three thyrotropic strains grow only in thyroidectomized mice, the thyroid was destroyed in all thyrotropic tumour hosts[2]. The adrenotropic tumours were maintained both in intact and adrenalectomized mice given deoxycortisone subcutaneously once a week.

The biological methods[2] and the methods for extraction of serum[3] used by this laboratory have been detailed elsewhere. Chromatographic separation of the iodinated compounds in tissues was done by the methods of Roche *et al.*[4]. 10–20 μc. T_4* (Abbott) repurified, when necessary, by paper chromatography and containing 0·4–1 μgm. stable T_4, were injected intraperitoneally and autopsy performed 4 hr. later. All tissue samples were rapidly weighed, counted for radioactivity to 5 per cent accuracy in a well-type scintillation counter and then frozen and lyophilized.

After chromatography, further identification of the radioactive compounds was made by two-dimensional

Table 1. Presence of T_3* in Paper Chromatograms prepared from Thyrotropic Mouse Pituitary Tumours 4 hr. after Injection Systemically of T_4*, but not from other Tumours or Control Tissue

Mouse strain	Tissue	Radioactivity in tissue/serum ratio	Range of values for percentage distribution of iodinated compounds on chromatograms		
			I*	T_4*	T_3*
LAF_1†	L23 thyrotropic tumour (autonomous, non-responsive)	0·1–0·3	17–54	18–53	28–30
	L24 thyrotropic tumour (autonomous, semi-responsive)	0·1–0·2	27–30	18–40	22–52
	4183 thyrotropic tumour (dependent, non-responsive)	0·1–0·2	4–35	23–65	31–42
	Adrenotropic tumour	0·2–0·3	29–30	70–71	0
	Fibrosarcoma	0·2	27	73	0
Swiss†	Mammary adenocarcinoma 755	0·1–0·2	43–44‡	56–57	0
LAF_1	Kidney	0·3–0·5	25–49	35–55	0
	Muscle	0·05	21	79	0

† See text for numbers of animals used.
‡ Corey, K. D., and Gross, J., *Cancer Res.*, **20**, 476 (1960), have reported formation of radiation iodide from T_4* in experimental mammary tumours.

chromatography, radioautography, and then elution and chromatography of the various radioactive areas.

The results obtained are shown in Table 1. Chromatograms of all three strains of thyrotropic tumours contained T_3* in equal, and high, concentrations, as well as T_4*, iodide and traces of radioactive 3,5,3′,5′-tetraiodothyroacetic acid. The adrenotropic tumours, however, contained no T_3*, and no T_3* was present in control tissues or in serum. A small amount, presumably of 3,3′,5′-triiodothyronine* (reverse T_3*), was demonstrated in chromatograms of serum and of some of the control tissues.

A specific alteration in hormonal environment is required to induce each of the secretory types of experimental mouse pituitary tumour. The resultant tumours are histologically homogeneous and specific. With dependent tumour strains, the same specific end organ hormone is required to suppress growth and hormonal secretion as is normally required to suppress pituitary secretion of the corresponding tropic hormone.

These close similarities to normal pituitary function suggest that each of the secretory pituitary tumours may have originated from corresponding specific anterior pituitary cells and thus reflect their functional behaviour. Ezrin has already postulated[5] from histological evidence that cells secreting thyrotropin and adrenocorticotropin are distinct and can be distinguished from the cells secreting the other hormones of the gland.

Since all three thyrotropic tumours of the present experiment contained a high proportion of T_3* after intraperitoneal injection of T_4* whereas the adrenotropic tumours contained none, the interesting possibility is raised that not only may there be a specific thyrotropin-secreting cell but also that it has a unique metabolic pathway for thyroxine. The formation of large amounts of T_3* could well be related to the inhibitory action which thyroxine exerts on thyrotropin secretion. Unfortunately, both dependent and autonomous thyrotropic tumour strains contained equally high proportions of T_3* and so any relationship to suppression of growth and hence to tumour autonomy or dependency seems to be eliminated.

It is possible that T_4* was metabolized to T_3* elsewhere in the body and was simply concentrated by the thyrotropic tumours. Predilection of the thyrotropic tumour for T_3* as opposed to the adrenotropic tumour would be interesting enough. However, no T_3* was identified in the serum or the various control tissues studied, and T_3* did not accumulate disproportionately in the thyrotropic tumours compared with control tumours and other tissues after T_3* injection[6].

Another possibility, that all pituitary cells deiodinate T_4* to T_3* but lose this system in the formation of adrenotropic tumours, seems unlikely. It is the metabolic behaviour of the thyrotropic tumours which is unique. Similarly, the possibility that the enzyme system responsible for direct diodination to iodide is lost in the thyrotropic tumours so that T_3* is formed alternatively seems excluded by the fact that T_3* is formed by the normal pituitary in about the same relative amounts[6].

This work was aided by Contract *SA*-43*ph*-2402, Cancer Chemotherapy National Service Centre, National Cancer Institute, Public Health Service.

Sidney C. Werner
Eugene M. Volpert
Raul Grinberg

Departments of Medicine and Biochemistry,
Columbia University College of
Physicians and Surgeons, New York.

[1] Furth, J., Burnett, W. T., and Gadsden, E. L., *Cancer Res.*, **13**, 298 (1953).
[2] Werner, S. C., and Grinberg, R., *Cancer Res.*, **21**, 522 (1961).
[3] Block, R. J., Werner, S. C., and Mandl, R. H., *Arch. Biochem. Biophys.*, **73**, 9 (1958).
[4] Roche, J., Michel, R., Jouan, R., and Wolf, W., *C.R. Acad. Sci., Paris*, **241**, 1880 (1955).
[5] Ezrin, C., and Nicholas, W. C., *Endocrinol. Soc. Forty-third Meeting Abs.*, 28 (1961).
[6] Volpert, E. M., Grinberg, R., and Werner, S. C. (unpublished results).
[7] Grinberg, R., Volpert, E. M., and Werner, S. C., *Endocrinol. Soc. Forty-third Meeting Abs.*, 63 (1961).

PHARMACOLOGY

A Micromethod for the Determination of Bradykininogen in Blood Plasma

Bradykininogen is the inactive precursor from which bradykinin, the hypotensive and smooth-muscle stimulating nonapeptide, can be released by the action of trypsin[1]. The concentration of the precursor in blood can be evaluated by the amount of bradykinin liberated by the action of trypsin on known volumes of plasma. The main difficulty found in a procedure for the determination of bradykininogen is the presence of trypsin inhibitors which interfere with the release of bradykinin, and the presence of enzymes which inactivate it in fresh plasma. This difficulty can be overcome if the plasma is previously heated in boiling water in the presence of diluted acetic acid, in order to obtain a denatured substrate. By this procedure trypsin inhibitors and bradykininolytic enzymes are inactivated[2,3].

After an examination of the conditions for the liberation of maximal amounts of bradykinin, a method for the determination of bradykininogen can be described as follows:

Venous blood is collected in a siliconed syringe moistened with 20 units of an anticoagulant ('Liquemine', Roche) and immediately transferred to a 15-ml. siliconed glass or polyethylene centrifuge tube. The blood is centrifuged and 0·2 ml. of plasma is transferred with a siliconed pipette to an 18 × 150 glass tube containing 1·8 ml. of 0·2 per cent acetic acid. The mixture is heated in a boiling water-bath for 30 min., cooled to room temperature in a tap-water bath, and is then adjusted with N sodium hydroxide (*c.* 0·06 ml.) to *p*H 7·30–7·80 (Merck Universal indi-

cator paper) and buffered at *p*H 7·8 with 0·5 ml. of 0·2 *M tris* buffer. The suspension formed is incubated with 0·2 mgm. of crystalline trypsin (Worthing Labs.), for 30 min. At the end of this period the enzyme in the clear digest is inactivated by adding 5 ml. of boiled alcohol. After immersion of the tubes for 5 min. into a water-bath adjusted at 70° C. the liquid is quantitatively transferred to a 125-ml. round-bottom flask and evaporated with reduced pressure to dryness. The dry material is suspended in 4 ml. of saline and assayed for bradykinin in a guinea pig ileum preparation using the 2 × 2 bioassay described by Rocha e Silva[4].

The bradykinin content does not change significantly if the blood is treated 2–4 hr. after collecting or if the dry digest is assayed after keeping it in an evacuated desiccator 12–24 hr.

The standard used for comparison was a preparation containing 3 units of bradykinin/mgm.; one unit of this preparation was equivalent to 0·5 μgm. of synthetic bradykinin (prepared by the Sandoz Labs.) according to the procedure of Boissonnas *et al.*[5]. One unit of bradykininogen is defined as the amount of the precursor able to liberate one unit of standard bradykinin or 0·5 μgm. of synthetic bradykinin.

Table 1 shows the concentration of bradykininogen found per ml. of blood plasma of man and other mammals measured according to the method described above.

Table 1. BRADYKININOGEN (UNITS/ML. PLASMA)

	Average	Range
Man	21·2	14·8–28·5
Ox	28·9	23·7–33·9
Horse	18·9	17·6–19·7
Sheep	12·7	12·0–13·8
Pig	8·1	5·5–11·6
Dog	12·3	12·0–13·0
Rabbit	20·1	17·5–22·7
Guinea pig	17·7	15·8–20·0
Rat	4·0	2·5– 5·0

Average of determinations made on the plasma of 10 men and 5 women (normals); other species, average of 3 animals.

The method described is being used in this laboratory for the examination of conditions expected to alter the bradykininogen content of plasma in several types of experimental shock, surgical conditions and pregnancy.

CARLOS R. DINIZ
IVAN F. CARVALHO
J. RYAN
M. ROCHA E SILVA

Departments of Pharmacology and Medicine,
University of São Paulo,
Ribeirão Prêto, São Paulo, Brazil.

[1] Rocha e Silva, M., Beraldo, W. T., and Rosenfeld, G., *Amer. J. Physiol.*, **156**, 261 (1949).
[2] van Arman, C. G., in *Polypeptides which Stimulate Plain Muscle*, edit. by Gaddum, J. H. (Livingstone, Edinburgh, 1955).
[3] Hamberg, U., and Rocha e Silva, M., *Arch. Intern. Pharmacodyn.*, **110**, 222 (1960).
[4] Rocha e Silva, M., *Acta Physiol. Lat.-Amer.*, **2**, 239 (1952).
[5] Boissonnas, R. A., Guttmann, St., and Jaquenoud, P. A., *Helv. Chim. Acta*, **42**, 1349 (1960).

Biological Spectrum of Some New Tuberculostatic 1,3,4-Oxadiazolones with Special Reference to Cross-Resistance and Rates of Emergence of Resistance

THE tuberculostatic activity of 5-(4-pyridyl)-1,3,4-oxadiazolone (*S* 57) has been repeatedly confirmed[1–3], and complete cross-resistance with isonicotinic acid hydrazid (isoniazid) in laboratory tests using *Mycobacterium tuberculosis* is reported by all workers in this field. It was, therefore, a little surprising to find that, whereas isoniazid is practically inactive against human leprosy, but active against rat murine leprosy, *S* 57 is highly active against both animal and human leprosy[4–6].

Although isoniazid and *S* 57 show complete cross-resistance in laboratory tests, yet if *H* 37 *Rv* is cultured in the presence of a concentration of isoniazid just sufficient to reduce, but not completely inhibit, growth, and a concentration of *S* 57 one-tenth of that required for growth inhibition, the rate of emergence of resistance to isoniazid is substantially reduced, showing that some difference in the biological spectra of isoniazid and *S* 57 is probably present[7,8]. This observation might have some bearing on the unexpected activity of *S* 57 in human leprosy, the more unexpected since it contains, in contrast to most leprostatics, no sulphur in the molecule.

The low toxicity and low local irritant properties of *S* 57 led to the extension of the above observations to the salicylate and *p*-amino-salicylate class of substance which shows reduced therapeutic usefulness owing to local irritant properties[9]. Conversion of the free carboxy group to the corresponding 1,3,4-oxadiazolones yields compounds possessing similar pharmacological spectra to the original salicylate and *p*-amino-salicylate, but showing, in fact as predicted, reduced toxicity and local irritant action. Thus 5-(2′-hydroxyphenyl)-1,3,4-oxadiazolone is an active anti-inflammatory, anti-pyretic and analgesic agent of low irritant properties and toxicity. 5-(2′-hydroxy-4′-aminophenyl)-1,3,4-oxadiazolone (*WS* 127) shows a similar tuberculostatic spectrum and order of activity as *p*-amino-salicylic acid, but is considerably more stable chemically. Here again complete cross-resistance to various strains of *Mycobacterium tuberculosis* has been observed in laboratory tests using strains resistant to *p*-amino-salicylic acid. Yet, on culturing *H* 37 *Rv* in the presence of both *p*-amino-salicylic acid and *WS* 127 at slightly subliminal concentrations the rate of emergence of resistance to both substances is substantially reduced compared with findings obtained using *p*-amino-salicylic acid or *WS* 127 individually on the same strain. The same phenomenon has been noted using *p*-amino-salicylic acid and 5-(2′-hydroxy-4′-aminophenyl)-1,3,4-oxadiazol-2-thione.

Thus, the laboratory cross-resistance tests commonly used apparently miss some of the finer details of activity, at least in this class of substance, which may be necessary to predict therapeutic effectiveness and rates of emergence of resistance both in animals and man.

A. E. WILDER SMITH

Department of Pharmacology,
School of Medicine,
University of Bergen, Norway.

HANS BRODHAGE

Department of Pathology,
Cantonal General Hospital,
Lucerne, Switzerland.

[1] Wilder Smith, A. E., *Science*, **119**, 515 (1954).
[2] Stempel, A., Zelauskas, J., and Aeschlimann, J. A., *J. Org. Chem.*, **20**, 412 (1955).
[3] Kakimoto, S., Krüger-Thiemer, E., and Wempe, E., *Arzneimittelforschung*, **12**, 963 (1960).
[4] Brodhage, H., *Leprosy Rev.*, 148 (1958).
[5] Jopling, W. H., and Ridley, D. S., *Leprosy Rev.*, 143 (1958).
[6] Bréchet, R., and Cochrane, R. G., *Leprosy Rev.*, (1961).
[7] Wilder Smith, A. E., *Arzneimittelforschung* (in the press).
[8] Wilder Smith, A. E., Brodhage, H., and Haukenes, G., *Arzneimittelforschung* (in the press).
[9] Matsumoto, K. K., and Grossman, M. I., *Proc. Soc. Exp. Biol. and Med.*, **102**, 517 (1959).

HÆMATOLOGY

Isolation of an α_2-Globulin from Human Plasma

While examining a method for the isolation of haptoglobin by chromatography on *DEAE* cellulose[1] we noticed that several α_1- and α_2-globulins were adsorbed under the experimental conditions used. One of these proteins, an α_2-glycoprotein, has now been obtained at about 90 per cent purity. The protein adsorbed together with haptoglobin, cæruloplasmin, cholinesterase and some α_1-globulins at *p*H 5·0 from human plasma diluted to a freezing point of − 0·15° is the last of these proteins to be eluted at the same *p*H but with increasing molarity of acetate buffer. Indeed, haptoglobin is eluted at 0·1 *M* acetate buffer, cæruloplasmin together with some α_1-globulin at 0·2 *M* and the unknown protein at 0·3–0·4 *M*.

When submitted to starch-gel electrophoresis[2], this protein migrates to the α_2-position. Protein π, as it may be called, is an α_2-glycoprotein containing about 10 per cent hexoses. Protein π is different from these three well-known α_2-globulins: haptoglobin, cæruloplasmin and α_2-macroglobulin. It differs from haptoglobin by its electrophoretic mobility on starch-gel, showing but a single band in the α_2-position (Fig. 1) and a somewhat faster migration on paper strip. Protein π has no hæmoglobin-binding capacity and no immunological relationships with haptoglobin. The new protein is precipitated by rivanol at *p*H 7, whereas haptoglobin is not. The protein under consideration differs from cæruloplasmin in its position on starch-gel at *p*H 8·6. It is colourless and contains no copper and shows no oxidase activity. There is no immunological relationship with ceruloplasmin as studied by the Ouchterlony technique and immunoelectrophoresis. Finally, there is no immunological relationship between α_2-macroglobulin and protein π and again the position on starch-gel of the two proteins is quite different.

The protein separated by chromatography on *DEAE* from whole plasma contains zinc in various amounts. Work is in progress to establish whether zinc is a definite part of the native protein, or a fortuitous contaminant. Protein π has been isolated under the same experimental conditions from fraction III as obtained during routine fractionation of human plasma following a slightly modified Nitschmann technique[3]. Whole plasma contains about 0·2 gm./l. of this protein and some total anti-human-serum gives a precipitation line with the purified protein. A screening for specific enzymatic or clotting activity has not yet given a positive result.

We thank Dr. Fine for immunoelectrophoretic analysis.

M. Steinbuch
J. Loeb*

Centre National de Transfusion Sanguine,
6 Rue Alexandre-Cabanel,
Paris.

* Chargé de recherches au Centre National de la Recherche Scientifique.

1 Steinbuch, M., and Quentin, M., *Nature*, **190**, 1121 (1961).
2 Smithies, O., *Biochem. J.*, **61**, 629 (1955).
3 Nitschmann, H., Kistler, P., and Lergier, W., *Helv. Chim. Acta*, **37**, 886 (1954).

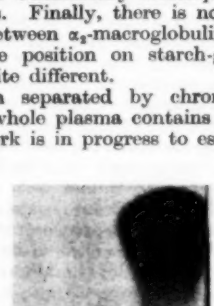


Fig. 1. Position on starch-gel of protein π (in the centre), whole plasma (right), isolated haptoglobin (left)

Quantitative Sub-Groups of the *B* Antigen in Man and their Occurrence in Three Racial Groups

Numerous authors[1–5] have described rare variants of the *B* blood group antigen which may differ qualitatively from other *B* antigens. Formaggio[4] concluded from his examination of 250 samples of *B* agglutinogens that differences within the *B* group were quantitative rather than qualitative. During the performance of 50 per cent end-point hæmagglutination assays of the *B*-anti-*B* system[7], it was noted that *B* cells from a Negroid individual were more strongly agglutinated than were those of Caucasoid origin. The probability–log assay curves in Fig. 1 show the difference observed with four doubling dilutions of anti-*B* serum in an equilibrium system with equal numbers of the two types of *B* cells of equal freshness. The percentages of cells agglutinated were obtained by the free-cell counting technique described in an earlier paper[8] and are plotted against the logarithms of the antiserum concentrations. Since the parallel assay curves indicated that these agglutinogens differed quantitatively rather than quali-

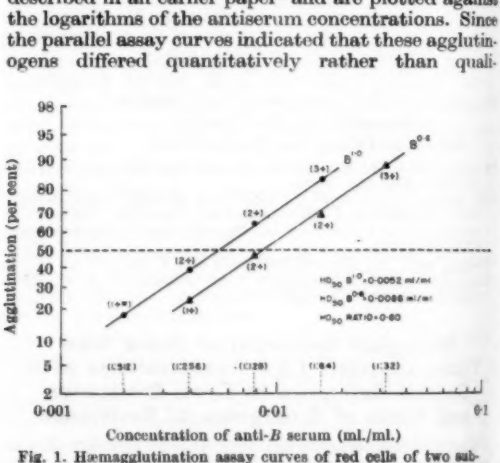


Fig. 1. Hæmagglutination assay curves of red cells of two sub-groups of the *B* antigen.
The degree of gross agglutination is indicated in parentheses. 3+, Large visible clumps of cells with only a few free cells; 2+, macroscopically many small clumps with more free cells; 1+, macroscopically no visible agglutination, microscopically many small clumps of 4–8 cells; 1+w, no visible agglutination macroscopically, microscopically small clumps of 2–4 cells

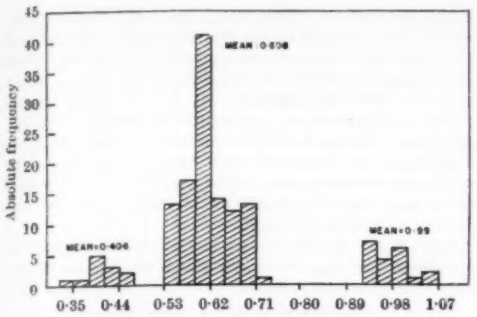


Fig. 2. Distribution of HD_{50} ratios for red cells of 143 blood group *B* individuals

tatively, the relative 'strength' of the *B* cells could be ascertained from the 50 per cent hæmagglutination end-points or HD_{50}'s interpolated from the assay curves. A ratio of the HD_{50}'s showed that the Caucasoid cell possessed only 60 per cent of the agglutinating strength of the Negroid cell. This difference was observed with numerous samples from these same individuals over a period of two years, and thus appears to be an intrinsic property of the agglutinogen from a given individual. The stronger cell was tentatively assigned a value of unity and provisionally designated $B^{1\cdot0}$ while the weaker Caucasoid cell was termed $B^{0\cdot6}$ to avoid confusion with the terminology B_{100}, B_{80}, B_{60}, etc., proposed by Hirszfeld[9,10] for the B_c (complete) antigen and its transition forms.

In order to determine whether quantitative subtypes vary in ethnic groups, samples of group *B* blood from Caucasian, Negro and Mongolian individuals were collected in equal volumes of modified Alsever's solution at the Washington Red Cross Blood Donor Center. The cells were compared with a standard $B^{1\cdot0}$ cell on the day of collection by the assay method. Concentrations of standard and unknown test cells were carefully adjusted to 26,000 ± 800 cells/mm.[3] by means of the Coulter counter. The frequency distribution of the HD_{50} ratio values for a population of 143 individuals of blood group *B* is shown in Fig. 2. It is evident that a segregation into three distinct populations ('percentage grades') was possible. The group of 12 samples which were more weakly reactive than the $B^{0\cdot6}$ group has been designated $B^{0\cdot4}$ since they exhibited approximately 40 per cent the agglutinability of the standard cell. The results of the testing of 100 Caucasoid, 25 Negroid and 18 Mongoloid *B* antigens are presented in Table 1. It is noted that $B^{1\cdot0}$ occurs in half the American Negro population but in only 9 per cent of Caucasians and in none of the Mongolians. The $B^{0\cdot6}$ predominates in Caucasians and Mongolians while $B^{0\cdot4}$ appears to be in highest frequency among Mongolian peoples. The fact that the results do not accord with the distribution expected of homozygotes (*BB*) and heterozygotes (*BO*) suggests that the observed differences are not related to the *ABO* genotype of the individual. In support of this, it was found that samples from one individual whose most probable genotype is *BB* as evidenced by family studies of three generations, and three from known *BO* individuals, fell within the same percentage grade ($B^{0\cdot6}$). Subjects were not available for examination of the heredity of these antigens; however, it is reasonable to assume that they are expressions of multiple allelomorphs at the *B* locus. These sub-groups may, indeed, correspond to the pleiades B_m, B_r, B_j described by Hirszfeld and Amzel[10]. On the other hand, $B^{1\cdot0}$ may be an exclusive character of the Negroid race since it is possible that the nine Caucasoid $B^{1\cdot0}$ antigens encountered are the result of race crossing. Whether $B^{1\cdot0}$ is an exclusive Negroid character or occurs in all races in different frequencies can only be resolved by testing pure racial populations.

Table 1. Distribution of *B* Antigens in Three Racial Groups

Racial group	Types of *B* antigen		
	$B^{1\cdot0}$	$B^{0\cdot6}$	$B^{0\cdot4}$
Caucasoid	8	87	9
Negroid	12	13	0
Mongoloid	0	15	3

Mary B. Gibbs
Joseph H. Akeroyd
Julia J. Zapf*

Department of Immunohematology,
Division of Communicable Disease
and Immunology,
Walter Reed Army Institute of Research,
Washington 12, D.C.

* Washington Regional Blood Program, Washington, D.C.

[1] Matta, D., Pub. No. 11, *Fac. Med., Univ. Egypt. Cairo* (1937).
[2] Mäkelä, O., and Mäkelä, P., *Ann. Med. Exp. Fenn.*, **33**, 33 (1955).
[3] Moullec, J., Sutton, E., and Burgada, M., *Rev. Hématol.*, **10**, 574 (1955).
[4] Levine, P., Celano, M. J., and Griset, T., *Sixth Cong. Intern. Soc. Blood Transfus., Boston*, 132 (1956).
[5] Dunsford, I., Stacey, S. M., and Yokoyama, M., *Nature*, **178**, 1167 (1956).
[6] Formaggio, T. G., *Z. Immunforsch.*, **110**, 1 (1953).
[7] Wilkie, M. H., and Becker, E. L., *J. Immunol.*, **74**, 192 (1955).
[8] Gibbs, M. B., and Akeroyd, J. H., *J. Immunol.*, **82**, 568 (1959).
[9] Hirszfeld, L., *J. Immunol.*, **55**, 141 (1947).
[10] Hirszfeld, L., and Amzel, R., *Rev. Immunol.*, **6**, 31 (1940).

A Correlation between Surface Charge and Coagulant Action of Phospholipids

The property of water-insoluble amphipathic molecules to alter the electrokinetic charge of lipids has been exploited by Bangham and Dawson[1] in elucidating the charge requirements of certain enzyme/substrate interactions. Their results show clearly the interdependence of both sign—positive or negative—and magnitude of enzyme and substrate interaction in any particular ionic environment. Whereas variation of *p*H, uni- or di-valent ion concentrations may affect the charge on both enzyme and substrate simultaneously, the addition of a water-insoluble amphipathic molecule to the lipid ensured an alteration of the substrate exclusively.

In the light of this experience and of the apparent anomalies in the ability of various phospholipid emulsions to accelerate the clotting of plasma[2–4], an attempt has been made to correlate the ζ potential of lecithin/amphipathic molecule mixtures with their effects on the clotting reaction promoted in plasma by Russel viper venom.

To 1·5 μmole-aliquots of ovo-lecithin in chloroform (isolated from mixed egg phospholipids by chromatography on alumina and silicic acid columns) were added progressive volumes of synthetic di-cetyl-phosphoric acid (0·66 μmole/ml.) in chloroform. Each mixture was taken to dryness *in vacuo* and emulsified in 1·0 ml. of a 0·045 *M tris* buffer in 0·1 *M* sodium chloride. Electrophoretic mobilities of the emulsion particles were measured by direct observation in a horizontal microelectrophoresis cell of the type described by Bangham, Flemans, Heard and Seaman[5]. The clotting system utilized fasting human plasma, obtained by centrifuging citrated blood

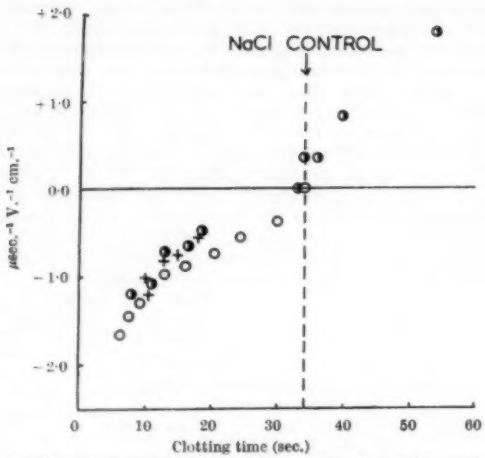


Fig. 1. Curves to show relationships between clotting time and electrophoretic mobility, positive or negative, of various lecithin mixtures. Mobilities were carried out in 0·045 *M tris* in 0·1 *M* sodium chloride at *p*H 7·2. Clotting times represent means of three measurements

○—○, 0·16 μmole lecithin plus varying amounts of dicetyl phosphoric acid; ◑—◑, 0·16 μmole lecithin plus 0·045 μ*M* dicetyl phosphoric acid to which is added varying concentrations of C_{22}-pyridinium bromide; ×—×, 0·16 μmole lecithin plus varying concentrations of phosphatidyl ethanolamine

first at 3,000 r.p.m. for 10 min. and then at 20,000 r.p.m. for 15 min. To 0·1 ml. of such plasma were added 0·1 ml. of the mixed phospholipid emulsion, 0·1 ml. of 1 : 100,000 'Stypven' brand (Burroughs Wellcome and Co.) Russel viper venom in water and finally 0·1 ml. 0·025 *M* calcium chloride. All reactants were maintained at 37° C. and the clotting reactions were followed at this same temperature.

Fig. 1 shows the relationship between the electrophoretic mobilities of the various lecithin/dicetyl phosphoric acid mixtures added to plasma and the observed clotting times. It can be seen that as the negative charge on the suspension increases, its ability to accelerate the clotting induced in plasma by Russel viper venon also increases.

The suspension promoting the most rapid clotting has a mobility of 1·6 sec.$^{-1}$ volt^{-1} cm.$^{-1}$. This agrees remarkably closely with the value for the particles present in the brain extract described by Bell and Alton[6] (Table 1). Lecithin/phosphatidyl ethanolamine mixtures are also negatively charged (due to the partial ionization of the amine group at *p*H 7·2) and the mobility/clotting time relationships coincide with those of lecithin/dicetyl phosphoric acid mixtures (Table 1 and Fig. 1).

That the surface charge is, in all probability, intimately concerned in the reaction is emphasized

Table 1. Comparison of Various Phospholipid Mixtures and Their Coagulant Action

Phospholipid mixture	Mobility (μsec.$^{-1}$ volt^{-1} cm.$^{-1}$)	'Stypven time' (sec.) av. of 3 readings
Tris/saline control	0	24
0·163 μmole lecithin	Iso-electric	24
0·115 μmole lecithin + 0·035 μmole dicetyl phosphoric acid	−1·6	7·0
0·034 μmole lecithin + 0·120 μmole phosphatidyl ethanolamine	−1·04	8·0
Brain phospholipids, Bell and Alton (ref. 6)	−1·69	7·9
Platelets	−1·2	8·7
0·163 μmole lecithin + 0·05 μmole dicetylphosphoric acid + 0·08 μmole C_{22}-pyridinium bromide	+1·8	34·0

by the observation that progressive neutralization of the negative charge of the suspension by the addition of C_{22}-pyridinium bromide to the lipid mixture results in a proportionately decreased effectiveness as evidenced by the prolongation of clotting time (Fig. 1). Addition of excess C_{22}-pyridinium bromide yields a positively charged lecithin/dicetyl phosphoric acid suspension causing an extended clotting time. This latter observation, too, holds important implications for the possible role of orientated surfaces in the whole process of blood clotting.

The effects on clotting time of other amphipathic molecules having alternative anions, for example, fatty acids, are at present under investigation. However, in view of the slightly different affinities of calcium ions for phosphate as compared with carboxyl anions, it is quite probable that the optimum mobilities of suspensions activated by fatty acid, in *tris*-buffered saline, may be different from those reported in Table 1.

Final appraisal of the relationships between the surface charge of orientated phospholipids and their possible catalytic role in enhancing blood clotting must await measurement of clotting time in the presence of a constant surface area of test lipid.

A. D. Bangham

Institute of Animal Physiology,
Agricultural Research Council,
Babraham, Cambridge.

[1] Bangham, A. D., and Dawson, R. M. C., *Biochem. J.*, **72**, 486 (1959).
[2] Poole, J. C. F., Robinson, D. S., and Macfarlane, R. G., *Quart. J. Exp. Physiol.*, **40**, 276 (1955).
[3] Poole, J. C. F., and Robinson, D. S., *Quart. J. Exp. Physiol.*, **41**, 31 (1956).
[4] O'Brien, J. R., *J. Clin. Path.*, **9**, 47 (1956).
[5] Bangham, A. D., Flemans, R., Heard, D. H., and Seaman, G. V. F., *Nature*, **182**, 642 (1958).
[6] Bell, W. N., and Alton, H. G., *Nature*, **174**, 880 (1954).

HISTOCHEMISTRY

Histochemical Examinations of Chymotrypsin-like Esterases

In 1953 Gomori[1] discovered a tissue enzyme which hydrolysed chloracetoxy naphthoic acid anilide. Mast cells were found to have a large amount of this enzyme activity. Based on the demonstration that the time required to achieve a given colour intensity in an enzymatic histochemical system is inversely proportional to enzyme activity[2], it was estimated that mast cells had 30 times the activity of liver and five times the activity of neutrophiles. Later, Benditt and Arase[3] were able to show that the enzyme in mast cells strikingly resembled bovine α-chymotrypsin. The present work has carried the resemblance further and examined the distribution of the enzyme in the rat, utilizing a newly synthesized series of esters of naphthoic acid anilide ('Napthol *AS*').

The presence of a phenyl group in the acidic portion of an ester is an established character of specific chymotrypsin substrates[4]. Esters of 'Napthol *AS*' were therefore synthesized beginning with the benzoxy ester and sequentially adding a methylene group between the carboxyl carbon and the phenyl ring, giving the phenylacetoxy, phenyl proprionoxy and phenylbutyroxy esters.

The use of diazo coupling with the free naphthol released by hydrolysis to measure enzyme activity in the test-tube was unsatisfactory because of: (1) the relatively slow coupling which precluded

satisfactory kinetic analysis; (2) the inhibitory effects of various lots of diazonium salts; (3) the low solubility of the azo compounds. The finding that free 'Napthol *AS*' exhibited fluorescence which was abolished by esterification and restored on hydrolysis provided the basis for a simple assay of chymotrypsin. 'Napthol *AS*' fluoresces maximally at 515 mμ when excited at 310 mμ. The excitation maximum coincides with an absorption peak that is absent in the esters. Self-quenching restricts the range of concentration over which fluorescence intensity is linearly related to concentration of the fluor. Fortunately the range of concentration for which the relationship is linear (0·5–15 μ*M*) is appropriate for the assay of enzyme activity.

Crystalline α-chymotrypsin was tested with each of the esters utilizing the fluorescent assay; the phenylproprionoxy ester was hydrolysed at an initial rate 10 times faster than the phenylbutyroxy and 30 times faster than either the benzoxy or phenylacetoxy naphthoic acid anilides. The *p*H optimum of 8·5 for the hydrolysis of the phenylproprionoxy ester agreed with the value obtained using *N*-acetyl tyrosine ethyl ester.

Rat tissues were examined histochemically for the presence of enzymes which would hydrolyse any of the esters. The standard method was to fix tissues in cold neutral buffered formol, washed with gum-acacia sucrose solution[5], and sectioned in the Pearse cryostat. Lipid was removed from the sections with methanol, and the sections incubated in the reaction mixture containing substrate at a concentration of 40 μ*M* in 40 per cent methanol in *tris* buffer at *p*H 8·0. Fast garnet *GBC* was used as the coupler. Sections were incubated 15 and 30 min. at room temperature, and then washed and mounted in polyvinylpyrrolidone[6]. Under these conditions, mast cells were the principal cell type to exhibit enzymatic activity in a variety of tissues examined, including liver, kidney, bone marrow, thymus and myocardium. The mast cell enzyme activity was parallel to that of chymotrypsin; the phenylproprionoxy ester was hydrolysed rapidly, the phenylbutyroxy slowly and the phenylacetoxy and benzoxy esters not demonstrably in the histochemical system. The mast cells from one dog mastocytoma and one cat mastocytoma also contained an enzyme which hydrolysed the phenylproprionoxy derivative.

In rat stomach and intestine, a population of cells distinct from the usual connective tissue mast cells evidenced esterase activity with the phenylproprionoxy and phenylbutyroxy compounds. These cells were usually smaller than connective tissue mast cells, with sparser granules staining less intensely with toluidine blue. Many of these cells appeared in the lamina propria while others were insinuated between epithelial cells of the mucosa in small intestine and colon.

Pancreatic zymogen granules which are known to contain chymotrypsinogen did not hydrolyse the phenylproprionoxy naphthoic acid anilide without prior activation of the enzyme precursor. Sections of pancreas were therefore treated with 0·05 per cent crystalline trypsin at *p*H 6 in the presence of calcium ion for 15–60 min. at room temperature. After trypsin activation, enzyme activity was evident in zymogen granules, in acinar lumina, in intralobular and interlobular ducts. The enzyme in the ducts was better demonstrated when methanol followed activation, whereas staining of enzyme in zymogen granules was greater if methanol preceded activation.

Two types of specificity are demonstrated in this work. There is the specificity of chymotrypsin and the related enzyme of mast cells for the phenylproprionoxy ester of a series of homologous 'Napthol *AS*' derivatives, and there is the restriction of the enzyme hydrolysing the phenylproprionoxy naphthoic acid anilide to connective tissue mast cells, intra-epithelial mast cells of the gastrointestinal tract, and pancreatic zymogen granules and intraductal secretion. The digestive function of pancreatic chymotrypsin is established; the presence of a chymotrypsin-like enzyme in connective tissue mast cells and intra-epithelial mast cells of the gastro-intestinal tract is a contribution of enzyme histochemistry yet to be exploited in work on normal and pathological function.

David Lagunoff
Earl P. Benditt

Department of Pathology,
University of Washington,
School of Medicine, Seattle, Washington.

[1] Gomori, G., *J. Histochem. and Cytochem.*, **1**, 469 (1953).
[2] Benditt, E. P., and Arase, M., *J. Histochem. Cytochem.*, **6**, 431 (1958).
[3] Benditt, E. P., and Arase, M., *J. Exp. Med.*, **110**, 451 (1959).
[4] Neurath, H., and Schwert, G. W., *Chem. Rev.*, **46**, 69 (1950).
[5] Holt, S. J., Hobbiger, E. E., and Pawan, G. L. S., *J. Biophys. and Biochem. Cytol.*, **7**, 383 (1960).
[6] Burstone, M., *Amer. J. Clin. Path.*, **28**, 429 (1959).

Histochemical Localization of Uridine Diphosphoglucose Dehydrogenase in Cartilage

This communication presents a method for the histochemical demonstration of uridine diphosphoglucose dehydrogenase in cartilage. This enzyme system is active in the pathway of synthesis of glucuronic acid[1,2].

Fifteen young adult albino mice on standard laboratory diet were killed by decapitation, and cartilage from various parts of the body, including larynx, trachea, ribs, joints and epiphyses were immediately removed. Fresh tissue blocks were frozen on dry ice. These were cut in a cryostat (−18° C.) with a rotary microtome at 16μ. The sections were placed on coverslips and rinsed for 5–10 min. in cold (4° C.) 0·1 *M* phosphate buffer at *p*H 8·4. They were then removed to an incubating mixture composed of 5 mgm. of the sodium salt of uridine diphosphoglucose, 2 mgm. diphosphopyridine nucleotide, and 2·5 mgm. of the paranitrophenyl substituted ditetrazolium salt ('Nitro-*BT*") in 5 ml. of 0·1 *M* phosphate buffer at *p*H 8·4. The incubation medium was warmed to 37° C. prior to introduction of the tissues, which were incubated for 30 min.–1 hr. The medium was then drained off and replaced by 10 ml. of 10 per cent neutral formalin at room temperature, in which the tissues were fixed for 1 hr. Sections were then mounted on slides in glycerogel and examined under the microscope.

In each experiment sections were also incubated in solutions which individually excluded substrate or coenzyme to control the specificity of the reaction. *p*-Chloromercuribenzoate (10^{-4} *M*) was used in some experiments as an inhibitor[1,3].

Uridine diphosphoglucose dehydrogenase activity was clearly demonstrated in cartilage cells from the larynx, trachea, ribs, joints and epiphyses. In all instances activity was most prominent in the cartilage cells located at the inner aspect of the perichondrium, and extended for only several cell layers in thickness

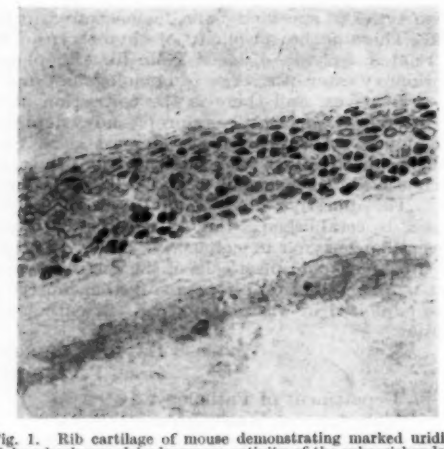


Fig. 1. Rib cartilage of mouse demonstrating marked uridine diphosphoglucose dehydrogenase activity of the subperichondrial cells. Perichondrium and intercostal muscle are negative. Incubation time, 60 min. (× 115)

(Figs. 1, 2). It was considerably reduced or absent in the more mature cartilage cells. The dye was deposited in a finely granular distribution throughout the cytoplasm; the nuclei were consistently negative. The controls, which lacked either substrate or diphosphopyridine nucleotide, were uniformly negative, thus excluding reduction of the ditetrazolium salt via oxidation of endogenous substrates or endogenous reductants. *p*-Chloromercuribenzoate (10^{-4} *M*) completely inhibited the reaction.

The mechanisms involved in the histochemical demonstration of oxidative enzyme activity by use of tetrazolium salts as indicators has been considered elsewhere[4]. In the system under discussion electrons are transferred from uridine diphosphoglucose to the coenzyme via a dehydrogenase and from the reduced coenzyme to the tetrazolium salt by a diaphorase. We are thus demonstrating the activity of a dehydrogenase–diaphorase system, both components of which must be acting to produce a positive result. The reduced tetrazolium salt forms a dark blue granular, insoluble, substantive diformazan at the site of enzyme activity. The

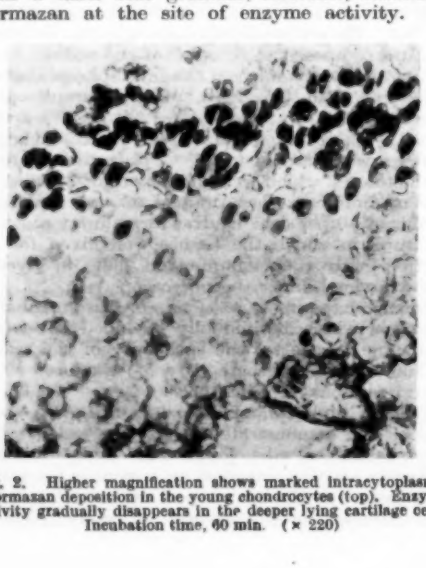


Fig. 2. Higher magnification shows marked intracytoplasmic diformazan deposition in the young chondrocytes (top). Enzyme activity gradually disappears in the deeper lying cartilage cells. Incubation time, 60 min. (× 220)

rinsing preceding incubation as outlined in the method is important in that it removes endogenous substances capable of reducing the tetrazolium salt independently. We have been unsuccessful in developing a histochemical method to demonstrate this enzyme system in the liver. The extreme diffusibility of some component of the system does not permit the rinsing which excludes or sufficiently minimizes non-specific reduction of the tetrazolium salt, a prominent effect in this organ at *p*H 8·4 (ref. 5). The enzyme in cartilage does not diffuse appreciably and thus is suitable for demonstration by this histochemical technique.

Glucuronic acid is important in intermediary metabolism, particularly in the conjugating mechanism of the liver (glucuronides), and as a component of mucopolysaccharides throughout the body[1,2]. There is evidence that the glucuronic acid available for these functions is synthesized from glucose via its uridine diphosphoglucose derivative[3,6]. Uridine diphosphoglucose is oxidized through a diphosphopyridine nucleotide-mediated dehydrogenase to uridine diphosphoglucuronic acid. The activity of this system in mammalian liver is well established[3]. Its role in the synthesis of hyaluronic acid in group *A Streptococcus* has been examined[7], and the presence of this system in murine mast cell tumour[8], and epiphyseal cartilage homogenates[9] from young rabbits, has been observed.

The distribution of uridine diphosphoglucose dehydrogenase in cartilage conforms to what we would expect in that it is most active in developing cells at the periphery which are presumably in the process of synthesizing new matrix. These cells would, therefore, require the greatest activity of an enzyme system which produces glucuronic acid for ultimate synthesis into the acid mucopolysaccharides of the matrix. The more mature central cells within well-developed ground substance may no longer require an active production of matrix components and show reduced or absent enzyme activity.

This method therefore demonstrates uridine diphosphoglucose dehydrogenase activity in the cytoplasm of murine cartilage cells from varying body sites. The synthesis of glucuronic acid available for incorporation into mucopolysaccharides probably takes place through the action of this enzyme system. The technique for its demonstration is based on the reduction of a tetrazolium salt.

This investigation was supported by a grant (*C*–3424) from the National Cancer Institute, U.S. Public Health Service.

Károly Balogh, jun.*
Richard B. Cohen
Department of Pathology,
Harvard Medical School and the
James Homer Wright Pathology Laboratories,
Massachusetts General Hospital, Boston.

* Postdoctoral Trainee in Pathology under U.S. Public Health Service Graduate Training Grant.

[1] Kalckar, H. M., and Maxwell, E., *Physiol. Rev.*, **38**, 77 (1958).
[2] Roseman, S., *Amer. J. Med.*, **26**, 749 (1959).
[3] Strominger, J. L., Maxwell, E. S., Axelrod, J., and Kalckar, H. M., *J. Biol. Chem.*, **224**, 79 (1957).
[4] Pearse, A. G. E., *Histochemistry, Theoretical and Applied*, 536 (Little, Brown and Co., Boston, 1960).
[5] Zimmermann, H., and Pearse, A. G. E., *J. Histochem. Cytochem.*, **7**, 271 (1959).
[6] Strominger, J. L., Kalckar, H. M., Axelrod, J., and Maxwell, E. S., *J. Amer. Chem. Soc.*, **76**, 6411 (1954).
[7] Markovitz, A., Cifonelli, J. A., and Dorfman, A., *J. Biol. Chem.*, **234**, 2343 (1959).
[8] Hambraeus, L., Rodén, L., and Boström, H., *Acta Soc. Med. Upsal.*, **44**, 247 (1959).
[9] Castellani, A. A., De Bernard, B., and Zambotti, V., *Nature*, **180**, 859 (1957).

IMMUNOLOGY

Mast Cell Lytic Antibodies

It is known that mast cell damage by antigen invariably occurs in those circumstances in which an anaphylactic release of histamine is produced in actively sensitized rats[1], mice[2] and guinea pigs[3]. The observation that in passively sensitized guinea pigs the mast cell reaction to antigen was rather uniform and graded with the dose of antibody[4] suggested that the large variation observed in actively sensitized guinea pigs could be due to variation in the relatively small amount of antibodies produced by them. Alternatively, some variation in the quality of the antibody was considered, for example, a difference between precipitating and non-precipitating antibody. However, early work[5,6] showed that precipitating and non-precipitating antibodies were equally efficient for passive sensitizing of guinea pigs.

I was led to the same conclusion by work on the behaviour of guinea pig mast cells in passive anaphylaxis[4]. However, some experiments with rats have suggested that in this species precipitating antibodies are not responsible for the mast cell damage and histamine release occurring in anaphylaxis, but that some other type of antibody might be responsible for these phenomena. For example, while on one hand mast cell damage and histamine release are very much increased in anaphylaxis of pertussis-treated rats[7] which produce very little, if any, precipitating antibodies, on the other both these phenomena may be much reduced in rats sensitized with Freund's adjuvant having considerable amounts of circulating precipitating antibodies. Actually, rats sensitized with Freund's adjuvant seem to be able to produce both types of antibodies since some of these animals present marked mast cell damage and histamine release when challenged with antigen, while others do not. However, this seems to bear no relation to the amount of precipitating antibodies produced by the animal. Thus, there is a difference between the amount of precipitating antibodies produced by immunized rats and the induction of mast cell damage by antigen in anaphylaxis.

Furthermore, I have not succeeded in duplicating mast cell damage and histamine release in anaphylaxis of rats passively sensitized with rabbit or even homologous precipitating antibodies. This occurred also when pertussis-treated rats were passively sensitized and shocked[8]. Therefore, it seems reasonable to conclude that some particular factor or type of antibody is required for the production of mast cell damage in rat anaphylaxis and that the anaphylactic syndrome produced in rats after passive sensitization with rabbit or homologous precipitating antibodies duplicates only that part of anaphylaxis due to precipitating antibodies.

As a working hypothesis we think that rat anaphylaxis is brought about by two different antigen–antibody reactions: (*a*) the reaction of antigen with precipitating antibodies—that do not cause mast cell damage; (*b*) the reaction of antigen with 'mast cell lytic antibodies', which are responsible for the anaphylactic release of histamine. It seems that rats produce normally both types of antibodies and that, depending on the condition of immunization, one of these types of antibody would predominate over the other. Thus, rats sensitized with Freund's adjuvant would produce predominantly precipitating antibodies while rats injected with *B. pertussis* along with the sensitizing dose of antigen would produce predominantly 'mast cell lytic antibodies'. However, the 'mast cell lytic antibodies' would never attain a concentration in the serum adequate to produce passive sensitization. This condition is necessary for explaining the repeated failure of serum taken from pertussis-treated rats to passively sensitize rat tissues either *in vivo* or *in vitro*. Furthermore, since the pharmacologically active substances released from the mast cells cannot account for the severity of anaphylaxis in pertussis-treated rats, one has to consider that the 'mast cell lytic antibodies' are not necessarily specific to these cells but are also very active in causing damage to other cells. Further experimental work is needed on this point.

I. Mota

Laboratory for Cell Physiology,
Department of Histology and Embryology,
Faculty of Medicine,
University of São Paulo, Brazil.

[1] Mota, I., thesis, University of São Paulo (1953); *Brit. J. Pharmacol.*, **12**, 453 (1957). Mota, I., and Ishii, T., *ibid.*, **15**, 82 (1960).
[2] Carter, P. B., Higginbotham, R. D., and Dougherty, R. D., *J. Immunol.*, **79**, 259 (1957).
[3] Mota, I., and Vugman, I., *Nature*, **177**, 427 (1956). Mota, I., *J. Physiol.*, **147**, 425 (1959).
[4] Humphrey, J. H., and Mota, I., *Immunol.*, **2**, 31 (1959).
[5] Kabat, E. A., and Boldt, M. H., *J. Immunol.*, **48**, 181 (1944).
[6] Benacerraf, B., and Kabat, E. A., *J. Immunol.*, **62**, 517 (1949).
[7] Mota, I., *Nature*, **182**, 1021 (1958).
[8] Mota, I., *Immunol.* (submitted for publication).

PATHOLOGY

Apparent Induction of Malignant Tumours by Organ-Specific Antiserum

It was reported[1] that rats injected with 3′-methyl-4-dimethylaminoazobenzene (3′-DAB) showed firm binding of this hepatocarcinogen to the structural lipo-protein complex forming the cytoskeleton, that is, endoplasmic reticulum of the liver cell. The binding here in terms of protein nitrogen was higher than elsewhere. It was shown similarly that firm binding occurred in a sub-microsomal fraction shown by Vogt[2] to contain all, or nearly all, the tissue-specific antigens in the liver cell. This fraction contained the bulk of the phosphatide in the microsomes and was therefore thought to be derived from the endoplasmic reticulum. As with the structural lipo-protein, binding was higher here than in any other cell fraction.

Two rabbits were immunized with 0·1 ml. of the sub-microsomal fraction prepared from the livers of rats injected intraperitoneally 48 hr. previously with 20 mgm. of 3′-DAB in arachis oil and two with a similar fraction from rats injected with arachis oil only. After nine injections (intravenous), the first with 1 ml. of Freund's adjuvant (intramuscular), tube-precipitin tests were erratic in regard to specificity but revealed precipitins to both fractions in all the undiluted sera. Absorption tests indicated that normal liver homogenate absorbed the precipitin from the control but not from the antiserum to the fraction from rats treated with carcinogen.

At this stage, 2-month-old Wistar rats (2 in each group) were injected (intraperitoneally) with pooled rabbit antiserum, absorbed for 1 hr. at 37° C. with normal rat kidney, spleen and lung homogenates, at a dose of 1 ml. spread over 15 injections in 7 months. Immunization of the rabbits providing the sera was continued for the first 9 weeks of this period. At 8 and 10 months after treatment ceased (that is, at the age

of 17 and 19 months), the 2 rats in the group treated with normal anti-sub-microsomal fraction sera died as the result of abdominal cancer. It is likely from the size and extension of the tumours that they had first appeared at a much earlier date. At 12 months after cessation of treatment, the rats injected with the 3′-DAB fraction appeared normal and were killed. No significant pathological lesions were found.

The first rat to die had a spherical tumour (3 cm. in diameter) adherent to the hilum of the liver with minute white foci throughout the liver. The peritoneum was riddled with small metastatic tumour nodules. The histology of the tumour was that of a typical bile duct carcinoma. Its origin was presumably in a branch of the common bile duct in the liver hilum. The liver itself showed very severe centrilobular necrosis with a cellular reaction and early periportal regeneration, but with many cells in this area still lysing. Some bile ducts in unaffected portal tracts appeared cancerous. The second tumour was quite different. It was very large (6 cm. × 5 cm.) and sited centrally in the posterior and upper abdominal wall. Its origin was obscure, but at autopsy the possibility of an upper para-aortic lymph node was considered. The whole of the liver surface showed minute yellowish-white foci. The tumour was highly malignant, consisting of large polyhedral cells with no definite acinar formation. Incorporated in it were areas of normal pancreatic glandular tissue. The liver parenchyma in this case was only mildly damaged, but the tumour was invading the liver subcapsular channels extensively. Many cells contained prominent blobs but did not stain for mucus. The nature of the tumour is unknown, but the possibilities include an undifferentiated pancreatic carcinoma and a lymphoid or reticulum cell sarcoma.

If we assume, as there is every reason from long experience with this rat strain, that these tumours were induced and not spontaneous, this result, in spite of the small numbers involved, is highly interesting for the following reasons. They are the first malignant tumours to be produced by the injection of antiserum. A tissue-specific antiserum has in one instance resulted in a tumour of a component of the tissue concerned, which would seem extremely unlikely to be a chance event. Not knowing the nature of the other tumour, there is no point in speculating at this stage on the mechanism of induction, except that it was very probably related to the treatment. Equally important is the fact that the antiserum to the carcinogen-treated liver fraction produced no tumours and no liver damage. There appears to be a striking parallel here with the results obtained by Price[3] at Leeds. She showed that treatment of kidney cells in tissue culture with 3 : 4-benzopyrene for 10 days rendered them incapable of inducing an antiserum nephrotoxic to mice *in vivo*. The inference was that the tissue-specific antigens had been modified antigenically by the carcinogen. It would appear that the hepatocarcinogen has also modified by haptenic binding the liver tissue-specific antigens and so made the induced antibody innocuous to normal liver.

The normal antiserum produced extensive liver damage in one case, and the process was still continuing at death long after cessation of treatment. There is a possibility here that an auto-immune reaction following liver damage had been established.

This experiment, although now of course being repeated in a larger and more detailed way, would seem to have greater significance, since it was planned on a purely theoretical basis in the hope of achieving the end which, at least in part, resulted. The postulate was that repeated exposure to tissue-specific antiserum would induce adaptive loss of tissue-specific antigens with malignancy as an essential consequence. If the results are repeatable on a larger scale, it would demonstrate beyond doubt that an immunological approach to carcinogenesis is valid, and that the immunological theory of the nature of cancer (see Green[4] for references) may be basically sound.

I am much indebted to Dr. G. M. Bonser for reporting on the tumour sections.

H. N. Green

Department of Experimental Pathology
and Cancer Research,
University of Leeds,
and Cancer Research Unit,
University of Sheffield.

[1] Westrop, P. W., and Green, H. N., *Nature*, **186**, 350 (1960).
[2] Vogt, P., *Nature*, **182**, 1807 (1958).
[3] Price, B. M., Ph.D. thesis, University of London (1961).
[4] Green, H. N., *Acta Unio Intern. contra Cancrum*, **17**, 215 (1961).

Susceptibility of Hamsters to the Oral Administration of *Mycobacterium johnei*

The development of a method of counting viable units of bacilli in the tissues of sheep[1] has enabled us to re-examine the potentialities of laboratory animals as experimental hosts for *M. johnei*. The oral route of challenge was chosen because it is the most likely route of infection in naturally occurring Johne's disease. Progressive infection in the tissues after oral dosing was considered to be a better test of the true susceptibility of animals than the direct introduction of bacilli by parenteral methods.

Three groups of twelve hamsters of about 20 gm. in weight were dosed orally on one occasion with 10^9, 10^8 and 10^7 viable units of *M. johnei* respectively. The strain of *M. johnei* was the sixth sub-culture from a clinical case of Johne's disease (bovine type) in a sheep. Five or six hamsters from each group were killed at 1 month and at 2 months after dosing. At these times the total viable unit counts were made of the spleen, the liver and the whole of the intestinal walls of each animal. The mesenteric lymph nodes and a piece of liver from each hamster were examined histologically. The results are shown in Table 1.

At 1 month after dosing, infections of the intestines were found in 1 of 6 hamsters given 10^7 viable units, in 3 of 6 given 10^8 units and in 5 of 6 hamsters given 10^9 units.

At 2 months the numbers of animals with intestinal infections were 1 out of 6, 6 out of 6 and 5 out of 5 at the 10^7, 10^8 and 10^9 dose-levels respectively. Where detectable infections were present in the small intestines the mean group counts at the 2-month examination were at least one $\log_{10}$ unit greater than those at the 1-month examination. This shows that progressive infections had been established. Infections were not progressive in the large intestines. *M. johnei* was recovered from the livers or spleens of all the hamsters given 10^8 and 10^9 viable units and examined at 2 months. The viable counts on these tissues were not significantly greater at the second examination compared with those obtained at the first. Whether the infection passed through the intestinal barrier at the time of dosing or as a result of secondary spread from primary progressive foci in the intestine is not yet known. At 1 month after

Table 1. VIABLE UNIT COUNTS IN THE TISSUES AND THE DISTRIBUTION OF MICROSCOPIC LESIONS IN THE MESENTERIC LYMPH NODES OF HAMSTERS

Time after dosing (months)	Dose (viable units)	Hamster No.	Counts expressed as $\log_{10}$ viable units* Spleen	Liver	Small intestine	Large intestine	Lesions in mesenteric lymph node
1	10^7	14	—	—	3·49	—	—
		13, 15–18	—	—	—	—	—
2	10^7	32	2·39	—	5·51	—	—
		33	3·09	3·39	—	—	—
		35	2·88	—	—	—	—
		31, 34, 36	—	—	—	—	—
1	10^6	7	4·22	3·92	—	—	—
		8	—	3·39	—	—	—
		9	—	—	3·79	—	—
		10	—	—	3·49	—	—
		11	5·35	6·08	4·35	3·49	+
		12	—	—	—	—	—
2	10^6	25	2·39	—	3·49	—	—
		26	4·66	3·65	4·49	4·54	+
		27	3·18	—	5·45	—	+
		28	4·08	2·88	5·74	—	+
		29	—	2·39	5·21	3·49	+
		30	4·54	—	—	4·58	+
1	10^5	1	—	2·39	4·49	4·45	—
		2	3·00	3·86	—	—	—
		3	5·29	5·04	4·98	4·20	+
		4	2·39	—	3·49	3·98	—
		5	3·95	—	3·98	4·10	—
		6	4·89	5·68	3·79	3·49	—
2	10^5	19	4·63	4·29	5·12	3·98	+
		20	4·32	3·70	4·88	—	+
		22	—	5·28	5·39	3·49	+
		23	5·18	4·43	4·54	3·79	+
		24	5·00	5·15	5·52	3·98	+

* The minimum detectable infections were $\log_{10}$ 2·39 viable units in livers and spleens and 3·49 viable units in intestines.

dosing lesions were present in the mesenteric lymph node of 1 hamster in each of the 2 groups given the higher doses. At 2 months, lesions were seen in 5 of 6 animals given 10^6 units and in all 5 hamsters given 10^5 units. No microscopic lesions were detected in the liver of any hamster.

Since there are already indications in sheep that vaccination may exert a beneficial effect by controlling the initial multiplication of *M. johnei* in the intestinal walls[2], the hamster is likely to prove a suitable experimental animal for the evaluation of vaccines against infection with *M. johnei*.

N. J. L. GILMOUR
J. G. BROTHERSTON

Animal Diseases Research Association,
Moredun Institute,
Gilmerton, Edinburgh, 9.

[1] Brotherston, J. G., Gilmour, N. J. L., and Samuel, J. McA., *J. Comp. Path.*, 71, 286 (1961).
[2] Brotherston, J. G., Gilmour, N. J. L., and Samuel, J. McA., *J. Comp. Path.*, 71, 300 (1961).

Behaviour of Protozoa under the Influence of the Synergetic Action of Polyethylene Glycol and Dyes

INVESTIGATIONS on trichomoniasis and the search for a remedy which would effectively destroy *Trichomonas vaginalis* are still being pursued. It has been difficult to find a preparation which is toxic to *Trichomonas vaginalis* and at the same time harmless as regards the vaginal mucous membrane.

Biological examination of the properties of polyethylene glycol showed that it fulfils these requirements[1]. Polyethylene glycol is available in both liquid and solid form depending on its molecular weight. The toxicity of this polymer decreases with increase in its molecular weight. Polyethylene glycol of 1,500–4,000 mol. wt. administered to experimental animals orally and intravenously for 2 years caused no pathological changes[2]. Intravaginal application of the polymer to rabbits did not bring about any deviations from the normal conditions in the vaginal mucous membrane[3]. In 40 hospital patients infected with *Trichomonas vaginalis* treated with the polymer satisfactory results were achieved in about 80 per cent of the cases[4]. The best results are obtained with aqueous solutions in concentrations of 25–50 per cent. For large-scale administration these concentrations are uneconomical. We decided, therefore, to ascertain whether a combination of the polymer with diamond green and gentian violet would not be more effective. It is known that these dyes are to a certain extent toxic in their action on *Trichomonas vaginalis*[5].

The lethal mechanism after administering polyethylene glycol is almost the same for *Paramecium caudatum* as for *Trichomonas vaginalis*. Since it is easy to culture and is very mobile, *Paramecium caudatum* was found to be more suitable for experimental purposes.

All the experiments were carried out as follows: Cultures of *Paramecium caudatum* were placed in a medium of egg-yolk and were used for the experiments when the density of the animals reached approximately 1,000 per 1 ml. of culture. Next, sterile aqueous solutions of polyethylene glycol of mol. weight 4,000, in concentrations of 1, 5, 10, 15, 20, 25 and 30 per cent, were prepared in 4 rows of test-tubes. To each row we added equal parts of:

(1) 0·1 per cent aqueous solution of diamond green
(2) 0·01 „ „ „ „ „ „ „
(3) 0·1 „ „ „ „ „ gentian violet
(4) 0·01 „ „ „ „ „ „ „

By means of a platinum loop, 1 drop of *Paramecium caudatum* culture and 1 drop of the appropriate polyethylene glycol solution with the dye, was placed on a concave glass slide. On mixing the liquids, the time when the *Paramecium* stopped moving was noted. This was taken to be the moment when the animals died, since afterwards they showed no sign of life even when replaced in the culture medium. Each experiment was repeated 5 times from which averages were calculated. Comparisons of these averages are given in Table 1. Investigations with the same solutions were carried out on *Trichomonas vaginalis* at the same time[6]. The results of this experiment are also given in Table 1.

Table 1. TIME REQUIRED FOR *Trichomonas vaginalis* AND *Paramecium caudatum* MOVEMENT TO CEASE AFTER THE ACTION OF POLYETHYLENE GLYCOL WITH GENTIAN VIOLET OR DIAMOND GREEN EXPRESSED IN HUNDREDTHS OF A MINUTE

	Concentration		Concentration of polyethylene glycol (per cent) 30	25	20	15	10	5	1
Trichomonas vaginalis	gentian violet	0·1	3·90	3·95	4·92	5·72	5·74	6·42	8·00
		0·01	7·32	9·74	11·12	17·30	21·50	35·20	38·80
	diamond green	0·1	4·47	4·74	4·82	5·76	8·26	11·63	13·72
		0·01	5·56	6·97	8·75	14·05	25·18	32·31	37·00
Paramecium caudatum	gentian violet	0·1	0·18	0·23	0·23	0·24	0·25	0·30	0·30
		0·01	0·34	0·39	0·41	0·54	1·70	2·90	3·18
	diamond green	0·1	0·23	0·25	0·26	0·28	0·29	0·31	0·32
		0·01	0·32	0·34	0·38	0·70	1·50	3·70	4·00

It may be concluded from our experiments that so far as these protozoa are concerned polyethylene glycol and the dyes have a synergetic action. On the grounds of our previous experiments and those described in this communication we consider that polyethylene glycol may be used for treating trichomoniasis. It is a non-toxic compound, cheap, and easy to apply. The addition of the dyes clearly has a more positive effect in the lower concentration of polyethylene glycol (1–20 per cent). This compound has a

stronger effect on *Paramecium caudatum* than on *Trichomonas vaginalis*.

Leopold Rejniak
Ludvik Komczyński
Department of Pathological Anatomy,
Medical Academy of Białystok,
Poland.

[1] Rejniak, L., *Pat. Pol.*, 7, 4, 397 (1956).
[2] McClelland, C. P., and Bateman, R. L., *J. Amer. Pharm. Assoc.* A.P. (1949).
[3] Komczyński, L., and Rejniak, L., *Pat. Pol.*, **10**, 3, 317 (1959).
[4] Komczyński, L., Rejniak, L., and Zomer, Y., *Pat. Pol.*, **10**, 2, 217 (1959).
[5] Okła, J., *Trichomoniasis vaginalis and its Treatment* (Warszawa, 1954).
[6] Komczyński, L., Kasprzycki, K., and Kasprzyk, H., *Roczniki AMB* (in the press).

BIOLOGY

Molybdenum and Symbiotic Nitrogen Fixation by Alder (*Alnus glutinosa* Gaertn.)

The specific effect of molybdenum on the nitrogen fixation by *Azotobacter* spp.[1,2], *Clostridium pasteurianum*[3,4], some blue-green algae (*Nostoc* and *Anabaena*)[5,6] and the symbiotic nitrogen fixation by legumes[7–9] is well established. So far, it is unknown whether molybdenum is also essential for the symbiotic nitrogen fixation of a non-legume such as alder (*Alnus glutinosa*). The aim of the present investigation was to seek such an effect.

The distribution of molybdenum in the different parts of an alder tree growing under natural conditions in a normal acid soil is shown in Table 1. From these observations it will be seen that molybdenum is accumulated in the root nodules. Except the seeds, all other parts show far lower molybdenum-levels. This result points to a molybdenum requirement for symbiotic nitrogen fixation in alder, since the root nodules are the seat of the nitrogen-fixation process.

To obtain more evidence that molybdenum is essential for the symbiotic nitrogen fixation by alder, growth experiments were performed in Neubauer glass jars containing 500 gm. of molybdenum-deficient low-moor peat soil containing ironstone. One set of pots received no treatment except a basic dressing of phosphate and magnesium, whereas a second received in addition 150γ molybdenum per pot. After a growth period of 6 months when the experiment was concluded, the alder plants on the molybdenum-deficient soil showed a pronounced reduction in development as compared with plants growing in the same soil, but with molybdenum added (Fig. 1). The molybdenum-deficient plants had pale-yellow leaves in contrast to the dark green colour of the leaves of plants supplied with molybdenum. In addition, the lower leaves of the molybdenum-deficient plants showed marginal scorch.

Table 1. Nitrogen and Molybdenum Contents of Different Parts of an Alder Tree growing in the Field* at Wageningen (Mansholtlaan), The Netherlands

Part of plant	Nitrogen percentage in dry matter	Molybdenum p.p.m. in dry matter†
Leaves (young)	3·84	0·25
Stems and branches	0·92 (0·73–1·11)	0·18
Roots	1·76	0·28
Nodules	2·37	1·71
Seeds	3·69	2·10

* The soil was a sandy loam (*p*H = 5·3) containing approximately 0·2 p.p.m. available molybdenum (*Aspergillus niger* method) (ref. 10).
† Determined chemically with potassium rhodanide, after reduction of any hexavalent molybdenum by stannous chloride.

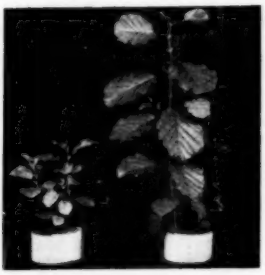


Fig. 1. Effect of added molybdenum on alder plants growing in molybdenum-deficient soil. Left, no molybdenum added; right, 150γ molybdenum as $Na_2MoO_4.2H_2O$ per pot

Obviously, the molybdenum-deficient plants suffered from nitrogen deficiency since yellow leaves and marginal scorch were found to be typical symptoms of nitrogen deficiency in non-nodulated alder plants growing in nitrogen-free molybdenum-containing nutrient solutions. Analyses showed that the molybdenum-deficient plants were considerably lower in nitrogen than those supplied with molybdenum (Table 2). The total amount of nitrogen in the tops of the molybdenum-treated plants was 370 per cent higher than that of control plants.

The molybdenum content of the various tissues of the molybdenum-deficient plants was extremely low (Table 3). Only in the root nodules was an appreciable amount of molybdenum found. The molybdenum-treated plants contained about 10–20 times more molybdenum in their tissues than molybdenum-deficient plants. The root nodules of the plants supplied with molybdenum contained 17·30 p.p.m. molybdenum in the dry matter, a value considerably higher than that known for legumes under comparable conditions[8].

The nodulation of the molybdenum-deficient plants was entirely different from that of plants grown in soil with an adequate molybdenum supply. In the latter plants mostly only one very big nodule (crown-nodule) was formed at the upper part of the root system, whereas in the molybdenum-deficient plants many small root nodules had developed which were uniformly scattered over the entire root system (Fig. 2). A similar effect has been observed by Mulder[9], who found that in molybdenum-deficient clover and lucerne plants the number of nodules was

Table 2. Effect of Molybdenum on Yield and Nitrogen Fixation by Alder Plants grown in Molybdenum-deficient Soil

Molybdenum* added (γ per pot)	Shoots: Mean shoot length (cm.)	Shoots: Fresh weight (gm. per pot)	Shoots: Dry matter (gm. per pot)	Total nitrogen in shoots (mgm. per pot)	Nitrogen (percentage increase)
0	18·2	11·2 ± 2·0	2·38 ± 0·64	46·4 ± 8·8	—
150	33·7	34·4 ± 0·1	7·58 ± 0·25	218·3 ± 8·6	370

* Applied as $Na_2MoO_4.2H_2O$.

Table 3. Molybdenum Contents of Leaves, Stems, Roots and Nodules of Alder Plants grown in Molybdenum-deficient Soil in the Absence or Presence of Added Molybdenum

Plant tissue	Molybdenum content p.p.m. in dry matter I*	II†
Leaves	< 0·01	0·27
Stems	0·14	1·89
Roots	0·24	2·62
Nodules	2·00‡	17·30

The molybdenum-deficient soil (*p*H = 5·2) from Groningen contained approximately 0·006 p.p.m. available molybdenum (*Aspergillus niger* method) (ref. 10).
* I, No added molybdenum.
† II, 150γ molybdenum (as $Na_2MoO_4.2H_2O$) supplied per pot containing 500 gm. soil.
‡ Approximate value since the sample was very small.

Fig. 2. Nodulation of a molybdenum-deficient alder plant showing many very small nodules uniformly scattered over the entire root system

much higher than in normal plants. Moreover, the same pattern of nodulation has been found in legumes inoculated with ineffective strains of *Rhizobium*.

Thanks are due to Prof. E. G. Mulder, who suggested this work, and to Dr. W. B. Deijs and Mr. O. C. Spoelstra for the chemical molybdenum analyses.

J. H. Becking

Laboratory of Microbiology,
Agricultural University,
Wageningen, The Netherlands.

[1] Bortels, H., *Arch. Mikrobiol.*, **1**, 333 (1930).
[2] Horner, C. K., Burk, D., Allison, F. E., and Sherman, M. S. J., *Agric. Res.*, **65**, 173 (1942).
[3] Bortels, H., *Zentralbl. Bakt.* (II), **95**, 193 (1936).
[4] Jensen, H. L., *Proc. Linn. Soc., N.S.W.*, **72**, 73 (1947).
[5] Bortels, H., *Arch. Mikrobiol.*, **11**, 155 (1940).
[6] Wolfe, M., *Ann. Bot.*, **18**, 299 (1954).
[7] Anderson, A. J., *J. Austral. Inst. Agric. Sci.*, **8**, 73 (1942).
[8] Mulder, E. G., *Plant and Soil*, **1**, 94 (1948).
[9] Mulder, E. G., *Plant and Soil*, **5**, 368 (1954).
[10] Hewitt, E. J., and Hallas, D. G., *Plant and Soil*, **3**, 366 (1951).

Œstrogen Content of Plants as a Function of Conditions of Culture

In the course of a broad investigation on the hormone content of plants, one of us[1], referring to the work of Alexander[2], stressed the important influence that conditions of culture could exert on the amount present in plants of substances having a hormonal action. Preliminary results of our investigation point to a definite influence in this respect.

Carrots cultivated over a period of several years on plots of soil that were left free from fertilizer and farm manure, or were treated either with a mixture of nitrogen, phosphorus and potassium, or with farm manure, were gathered and dried in the air at approximately 60° C., and tested for their œstrogen content, using immature female mice weighing 12–15 gm. The technique used was that described by Simon[3]. It is based on that used by Evans *et al.*[4], with the difference that both the carrot extract, and the diethylstilbœstrol used for the comparison, were given mixed in the diet. The diet was made up as follows: dextrine, 20; linseed meal, 13; barley, 10; wheat, 22; oats, 15; fish meal, 1·5; meat meal, 1·5; yeast, 0·5; mineral mixture, 2; gelatin, 2; dried milk, 12·5.

The food was prepared in the form of a biscuit of 0·5 cm. width.

The mice, divided into groups of six, were fed this diet during a 72-hr. period. They were then killed after 8-hr. fasting. The ovaries, Fallopian tubes and uteri were dissected out up to the cervices, stripped of all extraneous tissues, and weighed.

The findings are recorded in Table 1; they are given in equivalents of the doses of diethylstilbœstrol which served for comparative measurements in the control groups.

Table 1. Content, in Equivalent of Diethylstilbœstrol, of Carrots growing on Soil containing Various Fertilizers

Type of fertilizer	Content in equivalent of diethylstilbœstrol (γ/100)	
	Fresh product	Dry product
Control with no fertilizer	0·12–0·16	0·6–0·8
With nitrogen, phosphorus and potassium mixture	0·24–0·26	1·2–1·3
With farm manure	0·40	2

It is readily seen that, depending on the conditions of culture, the titre of œstrogenous substances in carrots can differ by as much as two-fold. Using the same procedure with wheat seeds, our figures were approximately 0·32 γ/100 of dry product of equivalent of diethylstilbœstrol for wheat growing on plots treated with nitrogen, phosphorus and potassium, and about 0·65 γ/100 for wheat growing on plots treated with farm manure.

R. Ferrando
M. M. Guilleux
A. Guerrillot-Vinet

Laboratoire de Nutrition et d'Alimentation,
Ecole Nationale Vétérinaire d'Alfort (Seine).

[1] Ferrando, R., and Ratsimamanga, A. R., *Ann. Nutrit. et Aliment.*, **14**, 331 (1961)
[2] Alexander, G., and Rossiter, S., *Austral. J. Agric. Res.*, **3**, 24 (1952).
[3] Simon, P. A., *Indian Vet. J.*, **36**, 426 (1960).
[4] Evans, S. J., Varney, R. F., and Koch, F. D., *Endocrinol.*, **28**, 747 (1941).

Incipient Diœcy in Pimento

Pimenta dioica (Linn.) Merrill, known as allspice or pimento, is an important Jamaican crop and the island is the world's chief supplier. Unfortunately, improvement of the crop is hindered by a characteristic type of unfruitfulness among certain trees. The problem has long been recognized and was mentioned by P. Browne[1] in 1755.

The genus *Pimenta* (Myrtaceae) is mono-specific and is of tropical distribution. Its flowers have four sepals and petals, many anthers and an inferior bicarpellary ovary enclosed in a receptacle. The single style is curved, the plane of the curve coinciding with the septum between the carpels. The specific name alluding to diœcy is of Linnæan origin and was based on herbarium material[2]. Preserved specimens of barren plants differ from bearing ones in that the former have a relatively conspicuous development of anthers.

Previous workers have proposed different explanations of barrenness. Grisebach[3] assumed the plant to be diœcious, the barren plants functioning as males. Browne[1], and more recently J. F. Ward[4], on the basis of the hermaphrodite floral structure assumed barrenness was due to some physiological disorder.

The Jamaican Department of Agriculture has had a number of pimento trees under observation for several years, and it is known that barrenness is a property of certain trees and not a transient feature such as

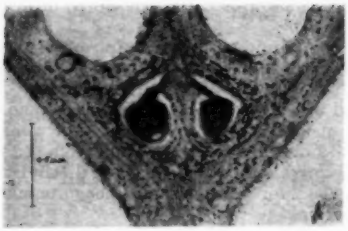


Fig. 1. Vertical section of barren receptacle

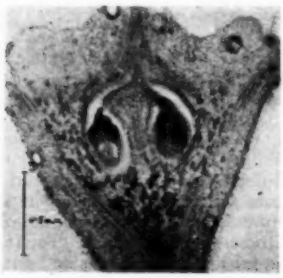


Fig. 2. Vertical section of bearing receptacle

biennial bearing. We have found consistent differences between barren and bearing trees, the flowers of the latter having fewer anthers. Anthers in five buds were counted from each of 30 trees in a random sample which was found to consist of 16 barren and 14 bearing trees. Barren and bearing trees had averages of 103·41 and 52·25 anthers per flower respectively.

Associated with high and low anther numbers were characteristic shapes of the receptacle. Figs. 1 and 2 show comparable sections through newly opened barren and bearing flowers. The former receptacle is seen to be relatively flat due to lack of development of tissue at the base of the style and the ovules are more nearly anatropous.

Flowers of barren trees were found almost always to fall after pollen shedding. Very occasionally one or two berries set but were in our experience invariably one-seeded. There was, however, an additional reason for non-abscission of barren flowers. Infestation by a gall midge (Cecidomyidae) caused destruction of ovules, but at the same time induced an irregular growth of the receptacle. Hitherto the gall midge has only been observed in the larval stage and a complete identification was not possible.

Pimento is a semi-wild crop, and the amount of deliberate planting is negligible. Apart from harvesting the only attention rendered in some groves is the removal of a few barren trees. Under such conditions, if barrenness were a genetic feature such as dicœcy, the two types might be expected to approach a one to one ratio with perhaps a slight excess of bearing trees. Table 1 shows a series of samples taken to examine this possibility.

Table 1. Frequency of Barren and Bearing Trees in Six Random Samples

Estate	Barren	Bearing	Total	χ^2 P	Remarks
Bengal	14	16	30	0·9–0·8	No barren trees removed
Montpeller	14	16	30	0·9–0·8	Removal of barren trees very infrequent
Grierfield	15	15	30	0·9–0·8	
Greencastle 1	17	13	30	0·7–0·5	A small proportion of barren trees removed annually
2	11	19	30	0·3–0·2	
3	9	21	30	0·05–0·02	
Total	80	100	180		

It was noticed that the barren stigma tended to disintegrate a few hours after flower opening, probably thereby hindering its pollination. Moreover, while it was possible to fertilize bearing plants with pollen from barren trees, inter-fertilization between bearing trees was not obtained. Since examples of the self-fertilization of bearing trees were found it was thought possible that the viability of bearing pollen declined more rapidly. Such a conclusion was supported by a series of pollen-culture experiments.

The present work suggests that pimento although structurally hermaphrodite shows, between barren and bearing trees, quantitative differences in the directions of maleness and femaleness respectively. Such a condition was considered by Darwin[5] to precede the establishment of a complete separation of sexes. It seems probable, therefore, that pimento shows, within the framework of a structurally hermaphrodite flower, an incipient dicœcism.

We wish to thank Mr. T. H. Farr, of the Institute of Jamaica, for assistance with the entomological aspect of this work.

G. P. Chapman

Botany Department,
University College of The West Indies,
Kingston, Jamaica.

S. K. Glasgow

Crop Agronomy Division,
Ministry of Agriculture and Lands,
Jamaica.

[1] Browne Patrick, *The Civil and Natural History of Jamaica*, 247 (1775).
[2] Merrill, E. D., *Contrib. Gray Herb.*, **165**, 30 (1947).
[3] Grisebach, A. M. R., *Flora of the West Indian Islands*, 240 (1864).
[4] Ward, J. F., *Pimento*, Min. Agric. and Lands (Jamaica) Pub. (1961).
[5] Darwin, C., *The Forms of Flowers*, Chap. 8, 281 (1877).

Circular Streaming Patterns in a Slime-Mould Plasmodium

In a previous publication[1], we suggested that the classical 'squeezing from behind' mechanism for protoplasmic streaming is inadequate to explain the actual movements observed in slime-mould plasmodia. As a result of a recent change in culture conditions, remarkably simple and direct evidence to substantiate this suggestion has appeared. Fig. 1 is a photograph of a plasmodium of *Physarum polycephalum* after two days growth at 22° C. in the dark on a particular growth medium. This medium contains, per litre, 20 gm. agar; 5 gm. 'Bacto-tryptone'; 5 gm. dextrose; 0·75 gm. 'Bacto-yeast' extract; 5 mgm. hæmin; 0·8 gm. potassium dihydrogen phosphate; 0·2 gm. disodium hydrogen phosphate; 100 mgm. calcium chloride; 100 mgm. magnesium sulphate; 200 mgm. citric acid; 10 mgm. ferrous chloride; 14 mgm. manganese chloride and 6 mgm. zinc sulphate, adjusted to *p*H 6·2 and autoclaved for 30 min. at 15 lb./sq. in. (This is modified from the formula kindly supplied to us by Dr. H. P. Eusch, University of Wisconsin.)

The noteworthy feature of this culture is the continuous circular 'vein' which traverses the plasmodium. This feature is observed repeatedly under these culture conditions.

In such a 'vein', all the streaming protoplasm may at times be seen to move continuously in a single

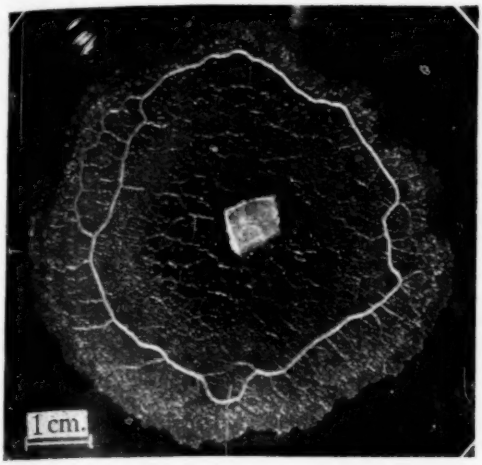


Fig. 1

direction, either clockwise or anti-clockwise. At other times various distributions of streaming in opposite directions in different parts of the main 'vein' are observed. We have observed no systematic correlation between streaming in the main 'vein' and streaming in the branches from it, nor between streaming at any point in the main 'vein' and any other point.

The existence of unidirectional streaming in such a continuous closed circuit, in the absence of any discernible valve- or ratchet-like mechanism, vitiates any hypothesis based on a spatial gradient of physical or chemical factors. On the positive side, this evidence seems to us to offer rather striking support for the notion that the mechanism which generates motive force must be distributed throughout the system and probably arises in the streaming material itself, not merely in the containing walls.

PETER A. STEWART
BABETTE T. STEWART

Department of Physiology,
Emory University,
Atlanta 22, Georgia.

[1] Stewart, P. A., and Stewart, B. T., *Exp. Cell Res.*, **17**, 44 (1959).

Production of Slime Substance in Mixed Cultures of *Rhodopseudomonas capsulatus* and *Azotobacter vinelandii*

WE have reported[1] that a symbiotic relationship exists between *Rhodopseudomonas capsulatus* (photosynthetic bacteria) and *Azotobacter vinelandii*. When they were mixed, the combination fixed three times more molecular nitrogen than did either pure culture. If the *p*H of the medium were controlled, the nitrogen fixation in the mixed culture was stimulated twelvefold[2].

Microscopically, the cells were surrounded by slime substances produced in the mixed culture after one month, but not observed in the pure culture. It seemed that these slime substances were produced by the interaction between the bacteria. Apparently the substances promoted nitrogen fixation in the mixed culture and also the growth of the mixed culture. Therefore, we extracted the slime substances and investigated them by paper electrophoresis.

van Niel's basal medium (without nitrogen and sodium bicarbonate) was used in this experiment, and 0·3 per cent sodium acetate, 0·2 per cent yeast extract and 0·2 per cent peptone were added to the medium. *R. capsulatus* and *Azotobacter vinelandii*, separately and in combination, were incubated in 3-l. flasks at 27° C. aerobically in the light (10,000 lux). After being cultured for 9 days the bacteria were collected, using a Sharples centrifuge, and washed twice with distilled water. The bacterial cells were stirred with 4 per cent sodium hydroxide solution for 2 hr. and then, after centrifugation, the supernatant was taken. This treatment did not appear to injure the bacterial cells because they remained very active, motile, and capable of good growth. Therefore, it was considered that the 4 per cent sodium hydroxide treatment exerted no effect other than to remove the slime substances from the outside of the cells. The slime substances were dialysed against distilled water at 2° C. for 7 days. The precipitate produced at that time was removed by centrifuging.

Fig. 1 shows the paper electrophoresis of the slime substances. It was found that a peak, labelled '*M*', was observed only in the mixed culture, and not found in either of the pure cultures of *R. capsulatus* and *Azotobacter vinelandii*. The slime substance was positive for ninhydrin, biuret, anthrone reactions and negative for the Benedict reaction. We conclude that the slime substance could be a kind of mucoprotein or mucopolysaccharide, produced specifically in the

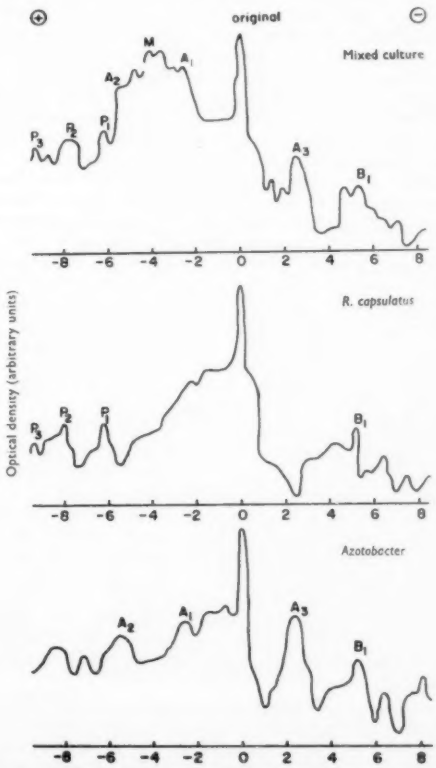


Fig. 1. Electrophoretic patterns of materials extracted from cell walls of *R. capsulatus* and *Azotobacter vinelandii* and their mixed cultures. Conditions: veronal buffer at *p*H 8·6; ionic strength, 0·05; Toyo No. 52 paper; 13 V./cm., 78 m.amp., 5 hr. Absorbancy measured with densitometer (Toyo-roshi Co.)

mixed culture and that it was a factor in promoting both growth and nitrogen fixation.

We have also found that treatment with sodium dodecyl sulphate[3] to extract extracellular deoxyribonucleic acid (DNA) resulted in the appearance of DNA in the slime substances. Further experiments to clarify the nature of these phenomena are being performed.

Azuma Okuda
Michiharu Kobayashi

Department of Agricultural Chemistry,
University of Kyoto, Japan.

[1] Okuda, A., Yamaguchi, M., and Kobayashi, M., *Soil and Plant Food (Tokyo)*, **6**, 35 (1960).
[2] Okuda, A., and Kobayashi, M. (unpublished data).
[3] Catlin, B. W., and Cunningham, L. S., *J. Gen. Microbiol.*, **19**, 522 (1958).

Seasonal Variation in Alginic Acid, Mannitol, Laminarin and Fucoidin in the Brown Alga, *Ecklonia radiata*

The brown alga *Ecklonia radiata* (Ag.) J. Ag. which occurs abundantly in the sub-littoral zone of the southern coasts of Australia, has been found to contain alginic acid, mannitol, laminarin and fucoidin, in conformity with observations on other species of the Laminariales from South Africa and the northern hemisphere. The above constituents were isolated from the seaweed and characterized by physical constants, chromatography and infra-red spectra[1]. An examination has now been made of the seasonal variation in the content of these carbohydrates, which is of interest in connexion with their biochemical function and possible utilization.

Samples were collected at Point Lonsdale, Victoria, each month during December, 1959–January, 1961. Each sample consisted of about twelve plants, which were dissected into laminæ, midrib and stipe. The 'midrib' was taken as that portion of the frond, comprising the elongation of the stipe, from which the 'laminæ' had been removed. The separate portions of the plant were milled through breaker plates in a Bauer defibrator and dried by azeotropic distillation with benzene. They were then ground in a Wiley mill to pass through a 100 mesh/in. sieve, and analysed according to the methods of Cameron, Ross and Percival[2]. In the case of the alginic acid determination, however, much more extensive acid washing was required than suggested by these workers in order to remove calcium ions from the alginate.

The maximum and minimum contents of the various components are shown in Table 1, with their time of occurrence. Marked variations were observed in the contents of laminarin, mannitol and alginic acid, the differences between the maximum and minimum contents decreasing in that order; however, the variations are less than those observed by Black and Dewar[3] for British *Laminaria* species. The fucoidin content is low and less variable relative to the total dry matter. It will be observed also that the contents of laminarin, and to a lesser extent mannitol, decrease from lamina to stipe, whereas the alginic acid content increases.

Laminarin is regarded by some investigators as a reserve carbohydrate. It is certainly stored in the frond, its content increasing to a maximum in summer and autumn, and decreasing rapidly to zero in mid-winter. The rapid decrease may be associated partly with the shedding of the lamina as well as utilization of reserve carbohydrate. The laminarin content may vary more widely in British species because of a slightly greater range in sea temperatures (45°–57° F. as against 54°–65° F.) and the considerably lower minimum temperature. In the case of laminarin and of alginic acid the seasonal fluctuations are similar for all parts of the plant. However, for mannitol the maximum and minimum contents are reached earlier in the laminæ than in the stipes, which seems to be more in accordance with the behaviour of a reserve carbohydrate than the variations observed for laminarin. The alginic acid content of *E. radiata* appears to be at a minimum in spring, at which time a maximum is reached in the British species.

Table 1. Seasonal Variation in Composition of *E. radiata*

	Maximum			Minimum		
	Lamina	Mid-rib	Stipe	Lamina	Midrib	Stipe
Alginic acid (per cent)*	24·5 April	28·4 April	30·2 April	17·0 September	19·0 September	19·8 September
Mannitol (per cent)*	16·6 February	16·7 April	13·6 June	8·5 July	7·4 August	6·7 October
Laminarin (per cent)*	9·8 May	7·0 May	1·1 May	1·0 August	0·0 August	0·0 August
Fucoidin (per cent)*	1·3 July	1·4 June	1·1 April	0·4 —	0·8 —	0·7 —
Dry matter (per cent)†	22 January	24 June	41 June	17 August	15 August	13 September

* Based on total dry material.
† Based on original wet weight.

From the point of view of possible utilization, *E. radiata* should be a useful source of alginic acid and mannitol and may yield appreciable quantities of laminarin, provided that due attention is paid to the seasonal variation.

We wish to thank Mr. K. J. Harrington for helping with the collection and preparation of samples, and Miss Glenys Chong for assistance in their examination.

C. M. Stewart
H. G. Higgins
Sue Austin

Division of Forest Products,
Commonwealth Scientific
and Industrial Research Organization,
South Melbourne.

[1] Stewart, C. M., and Higgins, H. G., *Nature*, **187**, 511 (1960).
[2] Cameron, M. C., Ross, A. G., and Percival, E. G. V., *J. Soc. Chem. Indust.*, **67**, 161 (1948).
[3] Black, W. A. P., and Dewar, E. T., *J. Mar. Biol. Assoc. U.K.*, **28**, 673 (1949).

Occurrence of *Phyllochaetopterus claparedii* McIntosh in the North-east Pacific

The waters near the Friday Harbor Laboratories of the University of Washington have been extensively dredged in recent years. It was of some interest, therefore, that a solitary *Phyllochaetopterus* of relatively large size was brought up from 22 fathoms over a muddy bottom just south of Orcas Island on August 26, 1960, with Forster's anchor dredge[1]. The specimen proved to be identical with the description of *Phyllochaetopterus claparedii* by McIntosh[2] found off Kobe, Japan. It has been recorded since only by Fauvel[3] from Cam Ranh and Tourane Annam (Vietnam). Treadwell's[4] specimens from Porto Rico are only doubtfully attributed to this species. The specimen from Friday Harbor had a pale yellow colour when alive apart from the grey-green gut, and was found in a transparent horny tube about 1 m. in length which was only faintly ringed (not jointed) distally and about 4 mm. across, comparable in size

with the largest specimens found by Fauvel from Cam Ranh.

I am indebted to E. and C. Berkeley for directing my attention to Fauvel's paper.

R. PHILLIPS DALES

Department of Zoology,
Bedford College,
London, N.W.1.

[1] Forster, G. R., *J. Mar. Biol. Assoc. U.K.*, **32**, 193 (1953).
[2] McIntosh, W. C., *Challenger Rep. Zool.*, **12** (1885).
[3] Fauvel, P., *Mem. Accad. nuovi Lincei*, Ser. 3, **2**, 279 (1935).
[4] Treadwell, A. L., *Scientific Survey of Porto Rico and the Virgin Islands*, **16**, Part 2 (New York Acad. Sci., 1939).

Oil Pollution of Marine Organisms

ALTHOUGH much publicity has been given to the harmful effects of oil on bird life, little evidence is available to show how marine organisms are influenced by oil pollution. Recent accidents involving the release of crude oil in Milford Haven afforded an opportunity to commence an assessment of the effects of this substance on the commonest intertidal animals and plants of a rocky coast.

It was found that even when the surface of the water is covered by a thick layer of oil, only a very thin film of oil is deposited on littoral animals and plants as the tide recedes, and much of this is washed off as the tide returns. Oil is, however, deposited at high-tide mark in the form of a band the width of which varies according to the degree of wave action on the shore at the time, and successive high tides may leave a series of bands of oil at slightly different levels. On very sheltered shores, each band may be only a few inches broad but on exposed shores the oil may be spread over several vertical feet of rock face. Clearly, therefore, the intertidal organisms most likely to be affected by oil pollution are those which occur between the lowest high water level of neap tides and the extreme high water level of springs, and include the acorn barnacles, *Elminius modestus*, *Balanus balanoides* and *Chthamalus stellatus*, the limpet, *Patella vulgata*, and on sheltered shores the algae *Fucus spiralis* and *Pelvetia canaliculata*.

Once deposition of crude oil has occurred, the lighter fractions gradually evaporate to leave a tarry residue which seems to be resistant to leaching by rain and moderate wave action. Erosion by water-borne particles (corrasion) probably occurs, but this is likely to be a very slow process except on shores subjected to intense wave action.

Examination of contaminated shores shortly after oil had been deposited showed that those acorn barnacles and limpets the shells of which had been covered by oil appeared to be unharmed and no barnacles were found in which the opercular plates had been glued together by the tarry residues. Examination a fortnight after deposition showed that the amount of oil remaining on surfaces in the immediate vicinity of limpets had been much reduced and that residual patches of oil situated a short distance from these animals exhibited the characteristic imprints of limpet radulas. It appeared likely that these molluscs were continuing to browse over the nearby barnacles and areas of bare rock in order to obtain food, but that in doing so they were inadvertently removing the layer of deposited oil. Since there was no decrease in the limpet population in the contaminated area, it seemed probable that the oil was non-toxic and that it was being passed through the alimentary canal and voided with the other faecal material.

Some confirmation that limpets are at least partially responsible for removing oil from contaminated shores was obtained during an examination of the latter six months after the accident. All traces of oil had been removed from the areas which support a population of limpets, but on one shore where oil had been deposited to a level above that of the uppermost limpets the tarry residue remained. Examination of barnacles which had been thickly smeared with oil showed that not only had all of it been removed but also that they had suffered no unusual mortality. Plants of *Pelvetia* and *F. spiralis* were also apparently unaffected and had continued to grow at a normal rate.

During the past two or three years, industrial emulsifiers have been increasingly used to clean up oil-polluted shores and they have been found to give effective results on sand and shingle beaches where the amenity value is of importance. Emulsifiers have also been used to clean up contaminated rocky shores, but it has been noticed that their use is followed by a severe mortality in intertidal organisms. Subsequent observations, as yet unpublished, have shown that the type of emulsifier most commonly used destroys all the limpets on a shore and 80–95 per cent of the acorn barnacles, and is extremely toxic to all other forms of intertidal life. It is suggested that in view of the fact that oil films are fairly quickly removed from rocky shores by natural processes, it is both wasteful and unnecessary to use emulsifiers on such shores.

M. GEORGE*

Dale Fort Field Centre,
Haverfordwest,
Pembrokeshire.

* Present address: Nature Conservancy, Government Offices, Bishopgate, Norwich.

MICROBIOLOGY

Solute Requirements for preventing Lysis of Some Marine Bacteria

PREVIOUS work has revealed a specific requirement of a number of marine bacteria for sodium for growth[1,2] and oxidative metabolism[2,4]. For optimum rate and extent of growth, 0·2–0·3 *M* Na^+ was required though limited growth could occur after a sufficiently long incubation period at as low as 0·03 *M* Na^+ (ref. 2). Since sucrose and K^+ exerted only a slight sparing action on the Na^+ requirement of the organisms examined it was concluded that the maintenance of an appropriate osmotic pressure in the medium was by no means the sole, or even the primary, function of Na^+ in the growth of the cells. Nevertheless, cells of many of the species lysed quickly when washed in distilled water, and in the case of one of the species examined in more detail, lysis occurred also when cells were suspended in solutions containing sodium chloride and potassium chloride at concentrations of these salts that were optimum for growth. Lysis, however, did not occur when 0·05 Mg^{2+} or Mn^{2+} was included in the suspending medium[3].

Since Mg^{2+}, and in some cases Ca^{2+} as well, are required for growth by all the species examined[5], the presence of these ions in the growth media used could possibly explain why no apparent osmotic requirements could be demonstrated during growth of the cells. Support for this conclusion was obtained when it was observed that cells of a number of

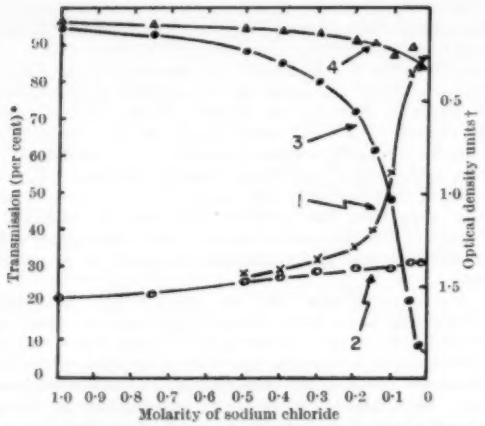


Fig. 1. Effect of concentration of sodium chloride on the turbidity of suspensions of a marine bacterium and on the absorption at 259 mμ of the supernates obtained from these suspensions in the presence and absence of Mg^{2+}. Curve 1, percentage transmission of bacterial suspensions in the absence of magnesium chloride; curve 2, percentage transmission in the presence of 0·05 *M* magnesium chloride; curve 3, optical density units of supernates from suspensions in the absence of magnesium chloride; curve 4, optical density units of supernates from suspensions in the presence of magnesium chloride

* 655 mμ filter, 1 *M* sodium chloride = 100.

† Calculated as optical density per ml. of supernate from the absorption of a suitable dilution at 259 mμ.

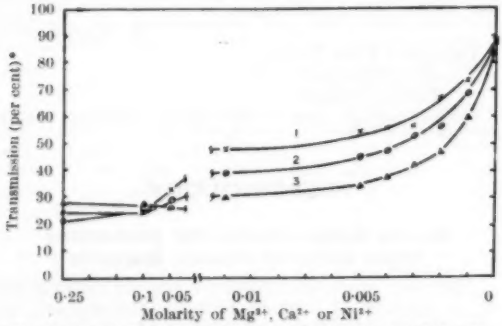


Fig. 2. Effect of the concentration of Mg^{2+}, Ca^{2+} and Ni^{2+} salts on the turbidity of suspensions of a marine bacterium. Curve 1, magnesium chloride; curve 2, calcium chloride; curve 3, nickel sulphate

* See Fig. 1.

marine bacterial species could be washed and suspended without apparent lysis in a solution of 1 *M* sodium chloride containing no added divalent ions. The quantitative requirements for various solutes for preventing lysis of several marine bacterial species have therefore been determined, and a preliminary report is presented here.

Organisms were grown in a nutrient broth–yeast extract medium containing salts previously established to be required for growth. Cells were collected by centrifugation at 4° C., washed twice in 1 *M* sodium chloride and finally suspended in a volume of the latter such that a 1:40 dilution gave about 20 per cent light transmission in a Coleman spectrophotometer equipped with a 655-mμ filter. A 0·25-ml. volume of cell suspension was then added to each of a series of tubes containing solutions of sodium chloride or other solutes to give a final volume of 10 ml.

The results obtained with organism *B*-16, a Gram-negative, polarly flagellated rod, tentatively identified as a species of *Pseudomonas* or *Spirillum*, are given in Fig. 1. Curve 1 shows that there is a small decrease in turbidity of the cell suspension (as indicated by the change in percentage transmission) between 1·0 *M* and 0·2 *M* sodium chloride and a very marked drop below 0·15 *M* sodium chloride. That the drop in turbidity below 0·15 *M* is due to cell lysis is indicated by the fact that turbidity cannot be restored by raising the concentration of sodium chloride. The change in turbidity is thus not merely an optical effect[1]. Furthermore no whole cells can be detected below the 0·15 *M* sodium chloride level by phase contrast microscopy. The same concentrations of lithium chloride and sucrose as sodium chloride and two to three times as much potassium chloride and glucose were also found to be capable of preventing lysis of these cells. The presence of 0·05 *M* magnesium chloride in the suspensions (Fig. 1, curve 2) prevented cell lysis at all concentrations of sodium chloride tested. When the suspensions at the various salt concentrations were centrifuged clear and tested for absorptive capacity at 259 mμ it was found (Fig. 1, curve 3) that the marked decrease in turbidity of the suspensions corresponded to a sharp increase in optical density of the supernatant solutions, indicating the release of nucleic acid material at low salt concentrations. The presence of 0·05 *M* Mg^{2+} in the suspending solutions largely prevented this release (Fig. 1, curve 4).

When the quantitative requirements for divalent cations for preventing cell lysis were examined it was found (Fig. 2) that as little as 0·001 *M* of such ions has a measurable capacity to prevent lysis while above this concentration the turbidity of the suspension was proportional to the concentration of ion added up to about 0·05 *M*. As Fig. 2 shows, Ca^{2+} and Ni^{2+} were more effective than Mg^{2+} in preventing cell lysis, while Mn^{2+} and Co^{2+} activities (not shown) lay between those of the latter. In general the order of activity of the divalent cations in preventing cell lysis appeared to be similar to that of their capacity to form chelate complexes.

Four other marine bacterial species have also been examined: another *Pseudomonas*, a *Flavobacterium* and two strains of *Photobacteria* (kindly made available by Dr. W. D. McElroy). Considerable species variation has been observed both in the capacity of cells to lyse at low sodium chloride concentration and in the ability of divalent cations to prevent it. It is possible to conclude in the case of all species examined, however, that Mg^{2+} and Ca^{2+}, the two divalent ions most prevalent in sea-water at their concentrations in the sea (0·05 *M* and 0·01 *M* respectively), would in all cases reduce and in some cases eliminate the requirement for other solutes such as Na^+ salts for preventing cell lysis. Complete details of these experiments will be published shortly.

Robert A. MacLeod
Tibor Matula

Department of Bacteriology,
Macdonald College,
McGill University, Montreal.

[1] MacLeod, R. A., Onofrey, E., and Norris, M. E., *J. Bact.*, **68**, 680 (1954).
[2] MacLeod, R. A., and Onofrey, E., *J. Cell and Comp. Physiol.*, **50**, 389 (1957).
[3] Tomlinson, N., and MacLeod, R. A., *Canad. J. Microbiol.*, **3**, 627 (1957).
[4] MacLeod, R. A., Claridge, C. A., Hori, A., and Murray, J. F., *J. Biol. Chem.*, **232**, 829 (1958).
[5] MacLeod, R. A., and Onofrey, E., *Canad. J. Microbiol.*, **3**, 753 (1957).
[6] Mager, J., Kuczynski, M., Schatzberg, G., and Avi-Dor, Y., *J. Gen. Microbiol.*, **14**, 69 (1956).

Dipicolinic Acid Content, Heat-Activation and the Concept of Dormancy in the Bacterial Endospore

Two distinct meanings have been applied to the use of the term 'dormancy' as applied to the bacterial endospore. First, the expression 'the dormant bacterial endospore' usually describes the apparent metabolic inactivity of the spore stage as compared with the vegetative cell. Secondly, the same term has been used to describe the fact that spore suspensions respond poorly or not at all to germination agents under conditions that permit rapid germination of aged[1] or heat-activated[2] spores. It is in the latter sense that the term dormancy is applied in this communication.

It is difficult to express quantitatively the dormant state of a spore preparation. Recent work suggests that the duration of heat-activation required for optimal germination can be used to measure the process. However, the duration of heat-activation necessary to induce optimal germination is not constant itself and can differ widely for different preparations of the same spore strain. The duration of heat-treatment necessary to induce optimal germination decreases with the age of the spore preparation[1]. L-Alanine-induced germination in *Bacillus cereus*[3] and glucose-induced germination in *Bacillus megaterium*[4] also indicate another relationship. With increasing concentrations of the germinating agents, less heat treatment is required for optimum germination.

In our work the requirement for heat-activation was further related to the chemical composition of the spore and the nature of the germinating agent employed.

Dipicolinic acid content and the germination-rate. The content of dipicolinic acid (DPA) in a spore can be varied by the conditions of growth and sporulation[5–7]. Also, it was observed that if higher concentrations of yeast extract were added to the '*G*' medium[5], the level of spore DPA could be increased from 7 to about 15 per cent of the dry weight. In the present work DPA contents of 2·2–15 per cent were employed.

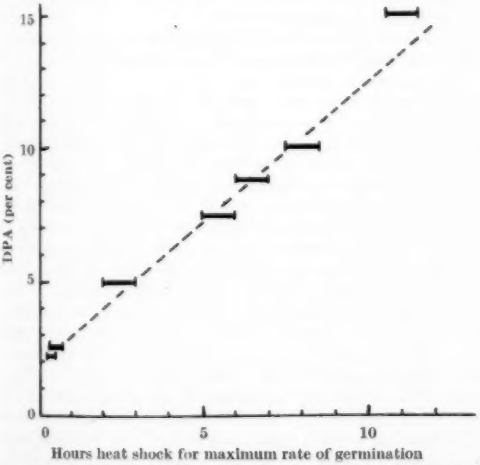


Fig. 1. Dependence of the duration of heat shock on intrasporal DPA concentration. Spores containing various concentrations of DPA were heat-shocked for various periods at 65° C., centrifuged, washed and resuspended in *M*/50 *tris* buffer *p*H 8 to an optical density of 0·40. Germination at 30° C. was initiated by the addition of 15 mgm./ml. L-alanine. The rate of germination was followed spectrophotometrically, as described by Woese *et al.* (ref. 8). The bars indicate the approximate time of heat-shock required to obtain maximum germination-rates

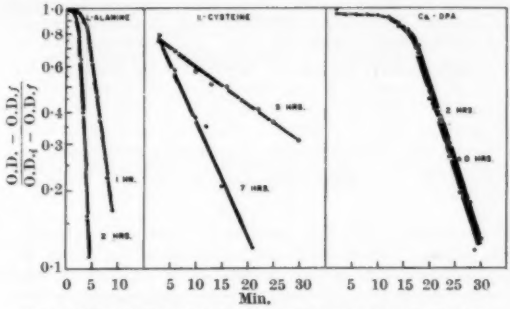


Fig. 2. Effect of heat-shock on the rate of germination in L-alanine, L-cysteine and CaDPA. See Fig. 1 for details of heat-shock and L-alanine-induced germination. L-Cysteine (15 mgm./ml.)-induced germination was followed at 30° C., whereas germination induced by $4·8 \times 10^{-2}$ *M* calcium chloride, 4×10^{-2} *M* DPA was carried out at 20° C. in the presence of 0·2 per cent gelatine

When the amount of heat-activation necessary to give optimal L-alanine-induced germination was determined it was found that the extent of heat treatment necessary (65° C. in water) was related to the DPA content. The heat-shock requirements increased with increasing contents of DPA. These results are shown in Fig. 1. The germination-rates obtained with the various spore preparations after optimal heat treatment were constant when the same concentrations of L-alanine were used.

Relationship between the heat-shock requirement and the germinating agent. The effectiveness of L-cysteine as a germinating agent under various conditions of heat-shock and with spores with various DPA contents was identical to that observed with L-alanine. On the other hand, the effect of calcium DPA was quite different. As observed by Rieman and Ordal[9], equimolar Ca^{++} and DPA are effective germinating agents for bacterial spores. The rate of germination in the presence of CaDPA was not influenced by heat-shock treatment or by the endogenous level of DPA. These results, as well as the behaviour of L-alanine and L-cysteine towards heat-shock treatment, are shown in Fig. 2.

DPA release during heat-activation. Harrell and Mantini[10] observed a release of DPA during the heat-activation of spores of *B. cereus* strain *T*. The hypothesis is therefore posed that germination ensues only after the endogenous DPA content is first reduced to a critical threshold. The observations that CaDPA complexes with amino-acids and peptides[11] and the numerous suggestions that dormancy is maintained by endogenous levels of inhibitors[12] are easily explained on the basis of a critical DPA threshold hypothesis. To test this directly with regard to DPA itself, the amount of DPA released after sufficient heat-shock to permit optimal germination was measured in spore stocks with widely varying levels of DPA. The results are shown in Table 1. In all spore stocks examined there was no relationship between the amount of DPA released and germination. Optimal germination occurred with about a 10 per cent loss of DPA. It is possible, however, that several reservoirs of DPA exist, only a minor fraction of which controls dormancy. Before such a possibility can be investigated, more information on the state of spore DPA will be necessary.

The ability to measure quantitatively the degree of dormancy of a bacterial endospore enables one to

Table 1. Release of DPA from Spore Suspensions during Heating

DPA content of spore suspension (percentage dry weight)	Heat activation (hr.)	Percentage DPA released during heat activation
14·9	11	10
10·2	9	9
7·4	8	14
4·9	4	9

Spores were grown in modified *G* medium, and heat-shocked as described in Fig. 1. The spores were then centrifuged and the DPA content of the supernatant determined by the method of Janssen *et al.* (ref. 13).

study some of the principal factors regulating dormancy. First, what is the chemical nature of the mechanism regulating dormancy? Although this cannot be answered, the present experiments provide a suggestion that DPA may be a part of the system responsible for the dormant state. If the intrasporal concentration of DPA determines dormancy, the breaking of dormancy cannot be related merely to the removal of DPA. One possibility is that the breaking of dormancy involves an activation stage (heat-activation) and that the achievement of this stage requires the displacement of DPA (or a compound correlated with DPA) from some endogenous site(s).

Secondly, is the dormant state unique? As seen above, the degree of dormancy is dependent on the germinating substance used. For example, high DPA spores are dormant towards L-alanine, and L-cysteine, but not towards CaDPA. The concept of dormancy is, therefore, not a general one, characterizing the state of a given spore suspension, but is relative to the germinating agent.

This work was supported in part by a research grant from the Wisconsin Alumni Research Foundation and by a grant from the National Science Foundation (*B*–7150).

A. Keynan*
W. G. Murrell†
H. O. Halvorson

Department of Bacteriology,
University of Wisconsin,
Madison, Wisconsin.

* Israel Institute of Biological Sciences, Ness-Ziona, Israel.
† Commonwealth Scientific and Industrial Research Organization, D.W. Food Pres., Homebush, Australia.

[1] Powell, J. F., *J. Gen. Microbiol.*, **4**, 330 (1950).
[2] Curran, H. R., and Evans, F. R., *J. Bacteriol.*, **49**, 335 (1945).
[3] O'Conner, R. J., and Halvorson, H. O., *J. Bacteriol.* (in the press).
[4] Hyatt, M. T., and Levinson, H. S., *J. Bacteriol.*, **81**, 204 (1961).
[5] Church, B. D., and Halvorson, H. O., *Nature*, **183**, 124 (1959).
[6] Black, S. H., Hashimoto, T., and Gerhardt, P., *Canad. J. Microbiol.*, **6**, 213 (1960).
[7] Halvorson, H. O., *Bacteriol. Rev.*, **23**, 267 (1959).
[8] Woese, C. R., Morowitz, H. H., and Hutchinson, C. A., *J. Bacteriol.*, **76**, 578 (1958).
[9] Rieman, H., and Ordal, J., *Science*, **133**, 1703 (1961).
[10] Harrell, W. K., and Mantini, E., *Canad. J. Microbiol.*, **3**, 735 (1957).
[11] Young, J. E., *Canad. J. Microbiol.*, **5**, 197 (1959).
[12] Stedman, R. L., Kravitz, E., Anmuth, M., and Harding, J., *Science*, **124**, 403 (1956).
[13] Janssen, F. W., Lund, A. J., and Anderson, L. E., *Science*, **127**, 26 (1958).

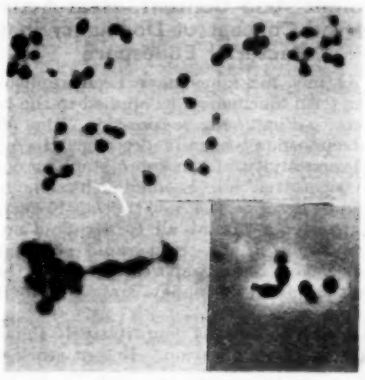


Fig. 1. Micrographs of bacteria. *a*, Single, pair and rosette forms; *b*, a chain form; *c*, budding bacteria. (× 1,500)

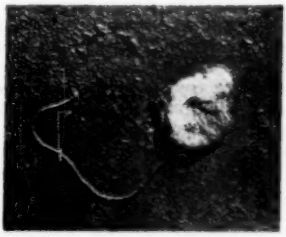


Fig. 2. A motile form possessing a single polar flagellum. Electron micrograph. (× 11,000)

Thermophilic Micro-organism of Uncertain Taxonomic Status from the Hot Springs of Tiberias (Israel)

In the course of a survey of the fauna and flora of the hot springs of Tiberias[1], which attain a temperature of 60° C., a bacterium was isolated which shows a special mode of reproduction and apparently has not yet been described.

The mature cells are spherical, ovoid or pear-shaped, measuring 0·5–2μ. They are non-pigmented, non-spore forming and Gram-variable. They occur singly or as free-floating groups of two, three, four or even more individuals, in the form of rosettes and short chains (Fig. 1 *a*, *b*). Daughter cells are formed as buds (Fig. 1 *c*) and appear sometimes to be situated between two larger cells. A low percentage of the solitary forms are motile, these cells possessing a single polar flagellum (Fig. 2).

Motility was observed occasionally in groups of two cells, and only in one case in a group of three.

The micro-organisms can be grown in autoclaved water from the hot springs, supplemented with 0·005 per cent yeast extract (Difco). No growth occurred when the concentration of the yeast extract was raised to more than 0·02 per cent. At 50° C. in the dark, the cultures achieved maximum cell density after one day, while at 37° C. they grow slowly and the peak is achieved only after several days. No multiplication occurs at 30° C. The organism is euryhaline and growth occurs over a range of 1–6 per cent (w/v) of total salt concentration; the concentration of minerals normally found in Tiberias hot springs is about 3 per cent[1]. Growth also occurred when spring water was replaced by sea water and supplemented with yeast extract as described here. The range of tolerance to sea water salinity is 0·5–8 per cent (w/v) total salinity. No growth was obtained on a solid agar medium. The highest concentration of agar ('Purified Bacto Agar, Difco') in which multiplication could be obtained was 0·2 per cent. The organism is aerobic as growth appears in a thin top layer of the semi-solid medium. The *p*H tolerance is wide, ranging from 6·2 to 9·5.

The taxonomic position of this micro-organism is as yet uncertain. It undoubtedly belongs to the

budding bacteria included in the order Hyphomicrobiales[3]. Unfortunately, the taxonomy of the members of this order is uncertain as most descriptions are based on microscopic examination[4,5].

Further investigations on this interesting micro-organism are being carried out.

DAVID KAHAN

Department of Zoology,
Hebrew University,
Jerusalem.

[1] Kahan, D., *Proc. Zool. Soc. Israel, Bull. Res. Counc. of Israel*, **9B**, 212 (1961).
[2] Buchman, M., *Tiberias Hot Springs*, Company ed. (1956) (in Hebrew).
[3] Bergey's *Manual of Determinative Bacteriology*, seventh ed. (The Williams and Wilkins Co., 1957).
[4] Henrici and Johnson, *J. Bact.*, **30**, 61 (1935).
[5] ZoBell and Upham, *Bull. Scripps Inst. Oceanography*, California, **5**, 239 (1944).

CYTOLOGY

Chromosome Preparation from Fresh and Cultured Tissues using a Modification of the Drying Technique

TECHNIQUES for making satisfactory chromosome preparations have evolved rapidly during the past decade since Makino and Nishimura[1] and Sachs[2] adopted pre-treatment with hypotonic solutions and the squash method of preparation. Administration of colchicine increased the number of countable mitoses and facilitated analysis by arresting cell division in metaphase, producing shortening of chromosomes and divergence of sister chromatids.

The Feulgen squash method[3] has been used widely for chromosome preparations. Although adequate in many instances it has several disadvantages. (1) Firm and even pressure is not easy to achieve, and areas of insufficient or excessive squashing are difficult to avoid. (2) Nuclei with incomplete or broken chromosomes are found in an appreciable number of cases. Tough *et al.*[4] reported 22 per cent of incomplete groups in their countable material. This complicates analysis. (3) Valuable material may be lost during squashing around the edges of the coverslip—an important factor, if small quantities only are available for analysis.

The drying technique for chromosome preparation was introduced by Rothfels and Siminovitch[5]. Spreading of chromosomes is achieved by alteration of surface tension forces involved during drying on the wet slide. The method has been reported on favourably by Moorhead *et al.*[6] and Tough *et al.*[4], the latter reporting that out of all countable groups 96 per cent were unbroken in their preparations. The technique was, however, only suitable for relatively fragile nuclei such as that from cultured human leucocytes. In human bone marrow and animal material, that is, bone marrow, spleen, lymph glands, etc., squashing was usually necessary.

A method of achieving satisfactory chromosome preparations by the drying technique has been evolved which can be applied to both human and animal tissues. Besides human cultured leucocytes, good-quality preparations have been obtained from marrow and spleen of mice, rats and rabbits. The technique is reliable, relatively simple, incurs minimal loss of material and produces a high count of unbroken and well-spread chromosome groups.

After pre-treatment with 'Colcemid' either *in vitro* in the case of cultures, or *in vivo*, a cell suspension is prepared (solid tissues are homogenized) and incubated with 0·95 per cent sodium citrate solution at 37° C. for 30 min. The following scheme is then followed. The suspension is centrifuged at 200*g* for 5 min. in Kahn tubes and the supernatant fluid is removed. Fixative (3 parts absolute alcohol to one part glacial acetic acid) is added and changed after 5 min. without disturbing the layer of cells lying at the bottom of the tube. The preparation is kept in iced water or a refrigerator at 4° C. for 1 hr. The fixative is removed and chilled 45 per cent acetic acid is added. The tubes are kept in a refrigerator for 18–24 hr. The 45 per cent acetic acid is removed and chilled 60 per cent acetic acid is added and the preparation kept cool for 1 hr. Only then are the cells resuspended in the 60 per cent acetic acid, so as to make a concentration of approximately 15×10^6 cells per ml. A microscope slide is placed on a flat piece of solid carbon dioxide and kept in place until a white film of frozen condensation forms on its upper surface. The slide is lifted and held obliquely until thawing has just begun at the edges. Small drops of the suspension are then placed on the frozen surface. The drops must not freeze on the slide but should spread rapidly, the cells adhering to the surface of the slide. The slide is then immediately heated over a spirit lamp, very gently if the material was obtained from cultured human leucocytes or more strongly in the case of other cells. The degree of heating may involve some trial and error, but in the case of mouse spleen cells, for example, it should be more rapid, so that drying is accomplished within several seconds, the temperature of the slide being above that tolerated by the back of the hand. Staining is performed with aceto-orcein.

The method produced satisfactory spreading of chromosomes in both fresh and cultured material. It was equally successful in preparations from marrow and solid tissues. The number of countable groups of chromosomes was considerably higher than in corresponding preparations by the squash technique. A large number of countable and clearly spread mitoses was obtainable on one slide. In the case of chimæric spleens from 3–10-day-old *A* strain mice (obtained by injecting spleen cells from adult *CBA* mice into *A* neonates), using a concentration of 15×10^6 cells per ml., it was possible to prepare 5–10 slides from each spleen and 100 or more clearly countable mitoses were usually present on each slide. This represented 20–30 per cent of nuclei in which chromosomes were visible. Ninety-four per cent of countable cells had the modal number of chromosomes.

In the technique described by Moorhead *et al.*[6], the final suspending medium before spreading was the fixative. It was found that the fragility of the nuclei could be increased by using 45 per cent acetic acid, and even more by 60 per cent acetic acid. Although it was safe and desirable to leave the cells in the 45 per cent reagent for a prolonged period of time (usually overnight), contact with the 60 per cent acetic acid for longer than 1 hr. was avoided, otherwise excessive spreading and scattering of the chromosomes occurred and staining was impaired. The thin film of frozen condensation produced by cooling the slide with dry ice afforded good spreading, and immediately afterwards, adherence of the cells to the glass surface. Subsequent heating was important to complete the spreading of more resistant nuclei during drying.

We are grateful to Prof. M. F. A. Woodruff for his encouragement and advice, and to Dr. Donald Michie for his helpful criticism. One of us (M. F.)

carried out the work during the tenure of a clinical research fellowship from the Medical Research Council.

M. Fox
I. M. Zeiss

Department of Surgical Science,
University of Edinburgh.

[1] Makino, S., and Nishimura, I., *Stain Tech.*, **27**, 1 (1952).
[2] Sachs, L., *Heredity*, **6**, 357 (1952).
[3] Darlington, C. D., and La Cour, L. F., *The Handling of Chromosomes*, second ed. (George Allen and Unwin, Ltd., London).
[4] Tough, I. M., Court Brown, W. M., Baikie, A. G., Buckton, K. E., Harnden, D. G., Jacobs, P. A., King, M. J., and McBride, J. A., *Lancet*, i, 411 (1961).
[5] Rothfels, K. H., and Siminovitch, L., *Stain Tech.*, **33**, 73 (1958).
[6] Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M. and Hungerford, D. A., *Exp. Cell Res.*, **20**, 613 (1960).

Culture of Human White Cells using Differential Leucocyte Separation

Several variations of white blood cell culture methods for the purpose of human chromosome study are now in use. All these are derived from the method of Hungerford *et al.*[1]. We have used the original and some of the modified procedures, particularly that of O. J. Miller (personal communication). In our experience with these techniques, about 75 per cent of cultures produce adequate numbers of mitotic figures for examination, while the remaining 25 per cent grow poorly, if at all. It was our impression that most failures occurred in specimens containing large numbers of polymorphonuclear leucocytes. On incubation, such cultures became excessively acid, and large clumps of degenerating polymorphs appeared.

While visiting the laboratory of K.-E. Fichtelius at the Histological Institute in Uppsala in 1957, one of us (K. H.) observed a method for removing polymorphonuclear leucocytes from white cell suspensions. The method was used for a metabolic examination of lymphocytes (Fichtelius, K.-E., and Hallander, H., personal communication). The procedure utilizes the phagocytic properties of polymorphonuclear cells by permitting them to ingest iron particles. The iron and the cells containing it are then removed by a magnet. Similar methods for different purposes have been used by several other investigators[2–4].

We have incorporated this procedure into our culture method, and have since performed more than 150 consecutive cultures with absence of growth in only one case. Our present technique produces good mitotic-rates (Fig. 1) and well-spread metaphase plates suitable for analysis (Fig. 2). The procedure is as follows.

Draw 10–20 c.c. of venous blood into a heparinized syringe and transfer to a sterile tube. Place the tube in an incubator at 37° at a 45° angle for 30–60 min., until the red cells sediment. Transfer the plasma to a sterile container having a flat surface and dilute with two volumes of culture medium (medium 199 with antibiotics). Add approximately 0·5 gm. of very fine sterile iron powder and place on a shaker at 37° C. for 30–60 min. with the flat surface of container down. The shaker speed is approximately 100 strokes per minute. Stand the container on a strong magnet for 5 min., transfer the supernatant to a sterile tube, and place this tube on the magnet for an additional 5 min. Transfer the supernatant to culture vessels. After performing a cell count, dilute the cell suspension with culture medium to achieve a final concentration of 0·5–1·0 × 10^6 cells per ml. (These are now 95–99 per cent mononuclear cells.)

Fig. 1. Low-power view of growing mononuclear cells, showing mitotic figures swollen with hypotonic salt solution. Note nuclei of mononuclear cells in various stages of growth

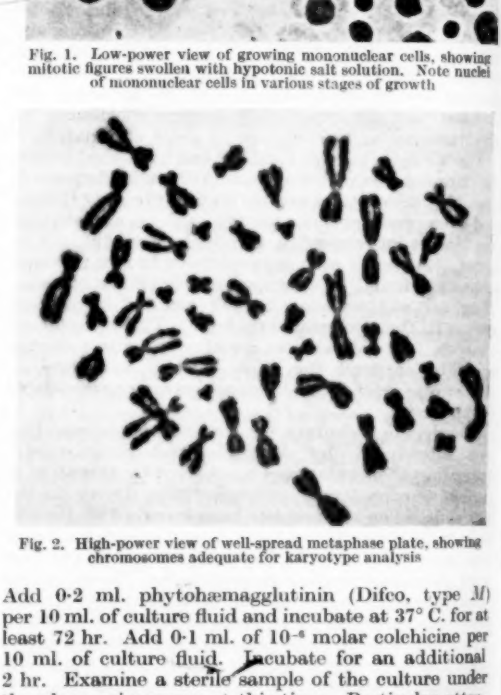


Fig. 2. High-power view of well-spread metaphase plate, showing chromosomes adequate for karyotype analysis

Add 0·2 ml. phytohæmagglutinin (Difco, type *M*) per 10 ml. of culture fluid and incubate at 37° C. for at least 72 hr. Add 0·1 ml. of 10^{-6} molar colchicine per 10 ml. of culture fluid. Incubate for an additional 2 hr. Examine a sterile sample of the culture under the phase microscope at this time. Particular attention is paid to clusters of mononuclear cells, which are examined for the presence of mitotic figures. If none is seen, incubate for an additional hour and repeat examination. When mitotic activity is observed, shake cultures well and centrifuge at 400 r.p.m. for 5 min. Suspend the cells in 1 per cent sodium citrate for 4 min. Add 1 drop of fixative (3 : 1 absolute ethyl alcohol–glacial acetic acid) and centrifuge at 200 r.p.m. for 2 min. Discard the supernatant. To the residue add the fixative very

slowly with constant agitation to a volume of 1 ml. After 5 min., centrifuge at 400 r.p.m. for 5 min.; resuspend in fresh fixative. (This may be repeated several times to improve chromosome spreading.) Place small droplets on a coverslip and air-dry by blowing. When dry, invert coverslip into a drop of 2 per cent acetic orcein on a slide and blot off excess stain for temporary preparation. For permanent preparations, immerse air-dried coverslips in stain for 1 min., dehydrate (tertiary butyl alcohol, 10 sec., twice; 1 : 1 tertiary butyl alcohol–xylene, 1 min.; xylene, 1 min., twice) and mount in 'Permount' or balsam. Such preparations are best examined by phase contrast. For bright-field examination, longer staining is required.

The technique outlined above depends for its success on the presence of a small amount of the substance phytohæmagglutinin, which appears to act as a mitotic stimulant[5]. Examination of growing cultures reveals that the mononuclear cells form large clusters, apparently as a result of the action of the phytohæmagglutinin, and it is only within these clusters that mitoses are found. That human mononuclear cells can be cultured *in vitro* without addition of phytohæmagglutinin is shown by the work of Timofejewsky in 1928 [6], where leucocytes from the buffy coat of centrifuged blood were grown in a plasma clot using autologous plasma. This suggests that part of the effectiveness of phytohæmagglutinin in causing leucocyte growth may be due to its ability to cause white cell clumping, providing an environment similar to that in a plasma clot.

This work was aided by grants from the American Heart Association, the U.S. Public Health Service (*H*–2202) and the New York City Health Research Council (*U*–1030).

JAMES HASTINGS
STANLEY FREEDMAN
ORLANDO RENDON
HERBERT L. COOPER
KURT HIRSCHHORN*

Department of Medicine,
New York University Medical Center,
550 First Avenue, New York, 16.

* Established investigator, American Heart Association.

[1] Hungerford, D. A., Donnelly, A. J., Nowell, P. C., and Beck, S., *Amer. J. Human Genetics*, **11**, 215 (1959).
[2] Cassen, B., Hitt, J., and Hays, E. F., *J. Lab. and Clin. Med.*, **52**, 778 (1958).
[3] Levine, S., *Science*, **123**, 185 (1956).
[4] Kuper, S. W. A., Bignall, J. R., and Luckcock, E. D., *Lancet*, **1**, 852 (1961).
[5] Nowell, P. C., *Cancer Res.*, **20**, 462 (1960).
[6] Timofejewsky, A. D., *Arch. Exp. Zellforsch.*, **6**, 259 (1928).

Two Types of Ribonucleoprotein in the Nucleolus of Intestinal Carcinoma of the Newt following Injection of Herring-Sperm Deoxyribonucleic Acid

RECENTLY I demonstrated two types of ribonucleoprotein in the nucleolus of tumour cells in the squamous-cell carcinoma of the lizard *Lacerta agilis*[1]. To establish whether a more general significance should be attached to this result I performed a corresponding investigation with the experimental carcinoma of the intestine of the newt *Triturus alpestris* following injection of herring-sperm deoxyribonucleic acid (eight days injection with 0·1 ml. of 5 per cent solution)[2]. After killing the animals on the 40th day of the experiment the nucleolar differentiation of the tumour cells was studied by the toluidine blue-molybdate method[3,4]. As in *Lacerta*[1], the ribonucleoprotein of the nuclear sap or parachromatin was coloured deep purple, the nucleolus partly assuming a green colour. Also in *Triturus* the existence of two types of ribonucleoprotein, if not actually demonstrated, had thus become highly probable. In the nucleoli a variable quantity of metachromatic ribonucleoprotein, as well as a number of very small vacuoles, appeared to be present, remaining unstained by the method described here. In the interphase cells the metachromatic ribonucleoprotein was most distinct, being in the form of small granules, hollow spheres or clusters of hollow spheres. Examination with a phase-contrast microscope showed that the nucleoli of the living tumour cells contained numerous very small vacuoles.

Comparing the nucleolus in the toluidine blue-molybdate stained sections with that in the living tumour cell it could be ascertained that the metachromatic ribonucleoprotein was situated around, or in, some very small vacuoles. After pre-treatment with ribonuclease or hot trichloroacetic acid[5] this nucleolar ribonucleoprotein was not coloured. In all phases of staining by the toluidine blue-molybdate method staining could be obtained in the same way as the nuclear parachromatin. Following Love and Bharadwaj[3,4] it can, therefore, in this intestinal tumour of *Triturus* also be called the nucleolar parachromatin. Just as in the squamous-cell carcinoma of the lizard and the Ehrlich ascites tumour[4], the tumour cells in *Triturus* often persisted throughout the mitosis, without being incorporated in the daughter nucleus. During mitosis the nucleolar parachromatin exhibited a different localization: in early prophase it was frequently situated at the rim of the nucleolus, to disappear from view during the later prophase, and become clearly visible again in the metaphase, anaphase and telophase as a vacuolated nucleolus green-coloured in the toluidine blue-molybdate sections.

Love[3] suggested that the nucleolar parachromatin is probably extruded from the nucleolus during the prophase to contribute to the accumulation of the granular parachromatin, which takes place in this period. The results obtained for the *Triturus* tumour may certainly be an argument for this reasoning. The earliest symptom of reformation of the nucleolus in the daughter nucleus was a metachromatic inclusion, afterwards acquiring an outer coating of ribonucleoprotein staining green in the toluidine blue-molybdate sections, which could be vacuolated as well as amorphous. This nucleolar differentiation of the tumour cells in *Triturus* is undoubtedly related to the special structures known for a long time already in the nucleolus.

The relationship of the two types of ribonucleoprotein in the nucleolus to the nucleolini[6–8] and the nucleonemata[6] could also be solved with the data obtained from the intestinal tumour of *Triturus*. Also in the tumour cells of *Triturus*, hollow and solid structures, resembling the true nucleolini, could be demonstrated after staining with iron hæmatoxylin. The physiochemical differences in ribonucleoprotein determining the differential staining by the toluidine blue-molybdate method may, therefore, also be responsible for some differences in the affinity for the hæmatoxylin. As these results are in good agreement with those obtained in the Ehrlich ascites tumour cells[4] and the squamous-cell carcinoma of *Lacerta*, it

would seem that they are certainly of general importance.

A. Stolk

Department of Histology,
Free University,
Amsterdam.

[1] Stolk, A., Thesis, Utrecht, 1950; *Proc. Kon. Ned. Akad. Wetensch.*, **56**, 157 (1953); *Nature*, **182**, 1177 (1958); *Naturwiss.*, **46**, 149, 654 (1959).
[2] Stolk, A., *Naturwiss.*, **47**, 88 (1960).
[3] Love, R., *Nature*, **180**, 1338 (1957).
[4] Love, R., and Bharadwaj, T. P., *Nature*, **183**, 1453 (1959).
[5] Serra, J. A., *Nature*, **181**, 1544 (1958).
[6] Montgomery, T. H., *J. Morphol.*, **15**, 265 (1899).
[7] Heidenhain, M., *Plasma und Zelle* (Gustav Fischer, Jena, 1907).
[8] Katable, C., and Sotelo, J. R., *Fine Structure of Cells*, 170 (Interscience Pub., Inc., New York, 1955).

PSYCHIATRY

Erythrocyte Cholinesterase-levels in Mental Patients

Rubin has published data[1a] on erythrocyte acetylcholinesterase which showed a bimodal distribution of this enzyme in schizophrenic patients. This paper was criticized[1b] and the criticism answered[1c]. Our work was an attempt to repeat Rubin's findings in larger groups of patients and normals.

The assay procedure we used is based on the following reactions:

acetylthiocholine + erythrocyte acetylcholinesterase → acetate + thiocholine

thiocholine + dithiobisnitrobenzoate → yellow colour

The rate of formation of the yellow anion of 2-nitro-5-thiobenzoic acid is proportional to the enzyme activity. This method is rapid and uses small amounts of blood (10 μl.) and is useful for surveys of this type. The plasma esterase was inhibited by addition of quinidine sulphate to the reaction mixture[2]. The assays reported in this communication are therefore of the erythrocyte esterase. Details of this method have been published[2].

Blood samples for the patient group were collected by the clinical laboratory staff from each patient on admission to this Institute. At that time the patient's record number and the date of the blood withdrawal were noted. A portion of the blood sample was assayed in our laboratories for erythrocyte acetylcholinesterase. At the time the assay was performed, the clinical diagnosis was unknown to us. After we had determined the erythrocyte acetylcholinesterase-levels of 64 patients, the diagnosis and status of these patients were studied. A psychiatrist (E. C.) examined all the hospital records on members of this group. The patients were then placed in the categories listed in Table 1 on the basis of the diagnosis recorded in the hospital record, supplemented by first-hand examination of the patients' chart. The results we obtained are shown in Table 1.

Table 1. Erythrocyte Acetylcholinesterase-levels

Type	$\bar{E}$	σ	n
Normal *A*	1·08	0·18	75
Normal *B*	1·01	0·16	8
Schizophrenic, no drugs	0·98	0·12	16
Schizophrenic, on phenothiazine	1·02	0·22	6
Organic psychoses and epilepsies	0·93	0·42	11
Psychoneuroses	1·00	0·23	13
Depressions	1·12	0·15	10

$\bar{E}$, Observed mean-rate (moles substrate hydrolysed per min. per red blood cell × 10^{12}).
σ, Standard deviation of the group.
n, No. in each group.

The group labelled Normal *A* is a sample of individuals who volunteered finger-tip blood during a display of medical research facilities held on this campus. The equipment for assaying the erythrocyte acetylcholinesterase was on display and the assays were performed in public. Persons whose blood was taken by our clinical laboratory and who were not patients (for example, physicians, nurses, staff, students, etc.) are included in the classification Normal *B*.

The results indicate that we cannot distinguish hospitalized mental patients from non-hospitalized, presumably normal persons, on the basis of mean erythrocyte acetylcholinesterase rates. The group called 'psychoses and epilepsies' did show considerably greater variability than the other groups. For this reason, we decided to follow the erythrocyte acetylcholinesterase rates for several days in these patients, to see if this difference was significant.

In summary, an epileptic patient was hospitalized for more than a month. During that period 28 erythrocyte acetylcholinesterase determinations were performed on his blood cells. Some of these were shortly prior to, others shortly after, seizures; some were quite distant in time before or after seizures. In addition, on two occasions we were able to make determinations at regular intervals after a seizure. In no case was there any obvious correlation of the duration or time of occurrence of the seizure with the observed erythrocyte acetylcholinesterase-level. Nor were his rates different from the normal (1·09 ± 0·12, Table 1) for the whole series. In a smaller series from two other such patients similar negative findings were observed. We believe, therefore, that the original observed large variability in this group of patients was an artefact of the small sample.

In re-examining Rubin's results it is clear that the major difference between his results and ours is the magnitude of the standard deviations observed. Our normals show about 15 per cent variation from the mean value. In a series of 101 human adult subjects, Sabine[4] showed a standard deviation of 10 per cent about her mean value. Sawitsky[5] reports a standard deviation of a group of 15 normal subjects which is 9·6 per cent of their mean activity. Rubin, on the other hand, observed a 3 per cent variation. Had his variation been as large as 10 per cent, his results would have been similar to ours.

The number of verifiable physiological differences between mentally ill patients and normals is small and any new observations in this field would be of value in assessing the etiology and progress of the disease. The difficulties of confirming the results of one investigation by another investigator in the field of mental health has been discussed by Kety[6]. We have been unable to confirm Rubin's results.

We wish to acknowledge the help and co-operation of Dr. Robert B. Anderson, Department of Neurology, University of California Medical Center.

George L. Ellman
Enoch Callaway

Langley-Porter Neuropsychiatric Institute,
San Francisco 22, California.

[1] *a* Rubin, L. S., *Science*, **128**, 254 (1958); *b*, Krech, D., Rosenzweig, M. R., and Bennett, E. L., *ibid.*, **128**, 1176 (1958). *c*, Rubin, L. S., *ibid.*, **128**, 1176 (1958).
[2] Ellman, G. L., Courtney, K. D., Andres, jun., V., and Featherstone, R. M., *Biochem. Pharmacol.*, **7**, 88 (1961).
[3] Wright, C. I., and Sabine, J. C., *J. Pharm. Exp. Therap.*, **93**, 290 (1948).
[4] Sabine, J. C., *Blood*, **10**, 1132 (1955).
[5] Sawitsky, A., Fitch, H. M., and Meyer, L. M., *J. Lab. Clin. Med.*, **33**, 293 (1948).
[6] Kety, S., *Science*, **129**, 1528 (1959).

FORTHCOMING EVENTS

Thursday, December 28

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 3 p.m.—Sir Lawrence Bragg, F.R.S.: "Electricity". The One Hundred and Thirty-second Course of six lectures adapted to a Juvenile Audience. Further lectures on December 30, January 2, 4, 6 and 9.)

Thursday, December 28—Friday, December 29

WORLD UNIVERSITY SERVICE (at B.M.A. House, Tavistock Square, London, W.C.1), at 10.30 a.m. on Thursday and 10 a.m. on Friday—Conference on "The Student and Mental Health".

Friday, December 29

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Dr. A. D. Booth: "Machines that Think" (Mann Lecture).

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

PHYSICIST (preferably with experience in isotope techniques, including autoradiography and physical methods for microanalysis) IN THE DEPARTMENT OF PREVENTIVE DENTISTRY, to engage in research into the mechanism of the action of fluorides in preventing dental caries, in co-operation with a research team including chemists and dental pathologists and histologists—Prof. J. L. Hardwick, Turner Dental School, The University, Manchester 13 (December 28).

LIBRARIAN—The Secretary, University of East Anglia, City Hall, Norwich, Norfolk (December 30).

JUNIOR LECTURER (interested in stratigraphy and sedimentation) IN GEOLOGY—The Registrar, Trinity College, Dublin, Republic of Ireland (January 1).

LECTURER or ASSISTANT LECTURER (medically or non-medically qualified) IN THE DEPARTMENT OF HISTOLOGY—The Registrar, The University, Liverpool, quoting Ref. No. CV/5/2/N (January 1).

LECTURER IN APPLIED MATHEMATICS, to take charge of the Electronic Computation Laboratory—The Registrar, University College, Singleton Park, Swansea (January 5).

LECTURER (medically qualified, and with special experience in histopathology) IN PATHOLOGY—The Registrar, Trinity College, Dublin, Republic of Ireland (January 15).

LECTURER IN GEOLOGY (Mineralogy and Petrology) at the University of New England, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 15).

LECTURER IN MICROBIOLOGY IN THE DEPARTMENT OF AGRICULTURE, University of Sydney—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 19).

SENIOR LECTURERS (2) IN ANATOMY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 19).

ASSISTANT LECTURER IN BOTANY—The Registrar, The University, Manchester 13 (January 20).

SENIOR LECTURER/LECTURER IN BACTERIOLOGY at the University College, Ibadan, Nigeria—The Secretary, Senate Committee on Colleges Overseas in Special Relation, University of London, Senate House, London, W.C.1 (January 22).

SENIOR LECTURERS or LECTURERS (with at least an honours degree or equivalent qualifications, with experience and research interests in pure mathematics) IN MATHEMATICS at the University of New South Wales—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2, and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, N.S.W., Australia (January 26).

LECTURER IN ENGINEERING, for duties which will include teaching and research in applied mechanics and one or more of the usual civil engineering subjects—The Secretary, The University, Aberdeen (January 31).

LECTURER IN PHYSICS—The Registrar, The University, Hull (January 31).

READER IN PHYSICS at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office,) Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 31).

RESEARCH FELLOWS (2) IN HYDROBIOLOGY, to work on fundamental problems in Lake Kariba at the Nuffield Kariba Research Station (University College of Rhodesia and Nyasaland)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (January 31).

LECTURER (with special qualifications or experience in inorganic chemistry or in some branch bearing on inorganic chemistry) IN CHEMISTRY at the University of Canterbury—The Registrar, University of Canterbury, P.O. Box 1471, Christchurch, New Zealand (February 28).

ROBERT BLAIR FELLOWSHIP IN APPLIED SCIENCE AND TECHNOLOGY tenable for one year of advanced study or research abroad—The Education Officer (WA.14/N/3316/12), County Hall, London, S.E.1 (March 12).

ASSISTANT BIOCHEMIST, Basic grade (preferably holding the A.R.I.C., the Grad.Mem.R.I.C. or B.Sc. (Hons.) in chemistry), in the Group Laboratory at Crumpsall Hospital—The Hospital Administrator, Crumpsall Hospital, Manchester 8.

ASSISTANT EXPERIMENTAL OFFICER (with a pass degree, H.N.C., or equivalent, or, if under 22, G.C.E. "A" level, O.N.C. or equivalent) IN THE CHEMISTRY DEPARTMENT, to help with research on the forms of combinations of magnesium, calcium and phosphate in cattle diets and ingesta, and on the development of the analytical methods used—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 61/15.

ASSISTANT EXPERIMENTAL OFFICER (with a pass degree, H.N.C. or equivalent, or, if under 22, G.C.E. "A" level, H.N.C. or equivalent) IN THE PHYSICS DEPARTMENT, for work on the flow and coagulation of blood—The Secretary, National Institute for Research in Dairying (University of Reading, Shinfield, Reading, quoting Ref. 61/19.

BRITISH PETROLEUM RESEARCH STUDENT (with experience in microbiological techniques) IN THE DEPARTMENT OF CHEMICAL ENGINEERING, for research on the conversion of petroleum hydrocarbons into fatty acids—The Secretary, University College London, Gower Street, London, W.C.1.

GRADUATE ASSISTANTS IN BIOCHEMISTRY—Dean, Faculty of Graduate Studies, University of Alberta, Edmonton, Alberta, Canada.

HEAD OF THE SCIENCE DEPARTMENT; HEAD OF THE MATHEMATICS DEPARTMENT; and ASSISTANT MASTERS TO TEACH SCIENCE, MATHEMATICS AND BIOLOGY—The Secretary, Atlantic College (U.K.), Ltd., Denmark House, 71/72 Piccadilly, London, W.1.

PHYSICIST, for teaching and fundamental research—Head of the Department of Physics, Victoria College, Victoria, B.C., Canada.

PHYSICS LABORATORY TECHNICIAN (man or woman, with experience of work in a teaching laboratory)—The Secretary, Royal Holloway College, Englefield Green, Surrey.

PRINCIPAL LECTURER IN CLOTH MANUFACTURE AND APPLIED TESTING; a SENIOR LECTURER IN TEXTILE PHYSICS, with particular emphasis on the structure of natural and man-made fibres; a SENIOR LECTURER IN GENERAL TEXTILES AND TESTING METHODS; a SENIOR LECTURER IN DESIGN AND COLOUR; a LECTURER (physicist or mathematician) for statistical work in textile engineering; and a LECTURER IN PHYSICAL CHEMISTRY, for the application of chemistry in textile processing—The Registrar, Bradford Institute of Technology, Bradford 7.

PRINCIPAL VETERINARY RESEARCH OFFICER (member of the Royal College of Veterinary Surgeons, or with an equivalent qualification) with the Federal Government of Nigeria—The Recruitment Attaché, Nigeria High Commission, 9 Northumberland Avenue, London, W.C.2, quoting Ref. Z.5/3.

SENIOR LECTURER IN AUTOMATIC CONTROL IN THE DEPARTMENT OF PRODUCTION TECHNOLOGY AND CONTROL ENGINEERING—The Secretary, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

SPECIALIST OFFICER (Plant Nematologist) (with a first- or second-class honours degree in natural science (botany or zoology) plus two years postgraduate experience) with the Federal Government of Nigeria—The Recruitment Attaché, Nigeria High Commission, 9 Northumberland Avenue, London, W.C.2, quoting Ref. B.16/2.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Science or Sanity: We Cannot Retain Both. By Thomas Brennan. Pp. 5. (Roydon, Harlow: Thomas Brennan, 7 Temple Mead, 1961.) [410

Society for Analytical Chemistry. Monograph No. 1: Methods for the Analysis of Non-soapy Detergents (NSD) Products. By G. F. Longman and J. Hilton. Pp. ii+30. (London: Society for Analytical Chemistry, 1961.) 15*s*. [410

National Central Library. 45th Annual Report of the Executive Committee, 1 March 1960 to 31 March 1961. Pp. 24. (London: National Central Library, 1961.) [410

Ministry of Agriculture, Fisheries and Food: National Agricultural Advisory Service. Experimental Husbandry Farms and Experimental Horticulture Stations—Progress Report 1961. Pp. v+55+4 plates. (London: H.M. Stationery Office, 1961.) 4*s*. net. [410

Universities Council for Adult Education. The Universities and Adult Education: a Report prepared by a Working Party of the Universities Council for Adult Education. Pp. 32 (Bristol: Mr. W. E. Salt, Hon. Secretary, Universities Council for Adult Education, 1961.) 2*s*. 6*d*. [410

University Grants Committee. Returns from Universities and University Colleges in receipt of Treasury Grant, Academic Year 1959–60. Pp. 76. (Cmnd. 1489.) (London: H.M. Stationery Office, 1961.) 4*s*. 6*d*. net. [410

West of Scotland Agricultural College. Research Bulletin No. 29: Comparison of Production from Three Varieties of Tall Fescue and Three Varieties of Meadow Fescue. By I. V. Hunt. Pp. 43. (Auchincruive, by Ayr: West of Scotland Agricultural College, 1961.) 1*s*. [410

Titles of Dissertations approved for the Ph.D., M.Sc., and M.Litt. Degrees in the University of Cambridge during the Academical Year 1960–1961. Pp. v+23. (Cambridge: Board of Research Studies, The University, 1961.) [410

Imperial College of Science and Technology (University of London). Calendar 1961–62. Pp. xi+396. Prospectus, 1962–63. Pp. viii+63+4 plates. (London: Imperial College of Science and Technology, 1961.) [410

Society for the Promotion of Nature Reserves. Handbook 1961: Forty-fourth Annual Report, year ending 31st March, 1961. Pp. 44+2 plates. (London: Society for the Promotion of Nature Reserves, British Museum (Natural History), 1961.) 10*s*. [410

Proceedings of the Royal Irish Academy. Vol. 61, Section A, No. 8: Statistical Efficiency of Different Methods of Estimation of Coefficients in a Very Simple System of Equations. By R. C. Geary. Pp. 67–76. 1*s*. 6*d*. Vol. 61, Section B, No. 14: The Lee Basin, Part 2: The Drainage Pattern. By S. Farrington. Pp. 233–254+plate 13. 2*s*. 6*d*. Vol. 61, Section B, No. 15: The Dalradian Successions and their

Relationship in the Churchill District of County Donegal. By T. C. R. Pulvertaft. Pp. 255–274+plate 14. 2s. 6d. Vol. 61, Section B, No. 16: The Effect of X-radiation on the Spermatogenesis of *Petrobius maritimus*. By R. S. Mathur. Pp. 275–282. 1s. (Dublin: Hodges, Figgis and Co., Ltd., 1961.) [410

Colonial Office. Fishery Publications, No. 16: A Quantitative and Qualitative Study of some Indo-West-Pacific Plankton. By J. H. Wickstead. Pp. iv+200+5 plates. (London: H.M. Stationery Office, 1961.) 55s. net. [410

The World Wildlife Fund. Save the World's Wildlife. Pp.20. (London: World Wildlife Fund, 1961.) [410

Manchester Literary and Philosophical Society. Memoirs and Proceedings, Vol. 103, 1960–61. Pp. 80+liii. (Manchester: Manchester Literary and Philosophical Society, 1961.) 31s. 6d. [910

Department of Scientific and Industrial Research: National Physical Laboratory. Notes on Applied Science No. 24: Photometric Standards and the Unit of Light. By J. 8. Preston. Pp. iv+32+4 plates. (London: H.M. Stationery Office, 1961.) 3s. net. [910

Ministry of Agriculture, Fisheries and Food. Fishery Investigations, Series II, Vol. XXIII, No 3: On Fecundity and Larval Abundance of Downs Herrings. By J. P. Bridger. Pp. ii+30. 10s. net. Series II, Vol. XXIII, No. 6: The Principles of Water Sterilisation by Ultra-violet Light, and the Application in the Purification of Oysters. By P. C. Wood. Pp. iv+48+4 plates. (London: H.M. Stationery Office, 1961.) [910

British Broadcasting Corporation. B.B.C. Engineering Monograph No. 38: Operational Research on Microphone and Studio Techniques in Stereophony. By D. E. L. Shorter. Pp. 24. (London: British Broadcasting Corporation, 1961.) 5s. [910

Planning, Vol. 27, No. 455, (October, 1961): International Money and Capital Movements in Europe. Pp. 241–280. (London: Political and Economic Planning, 1961.) 4s. [910

Astronomical Contributions from the University of Manchester. Series III, No. 79: Studies in Lunar Topography, VIII: A Catalogue of Measured Heights in the Regiomontanus and Hell Plain Regions of the Moon. By Geoffrey Turner. Pp. 34 (2 plates). (Manchester: The University, 1961.) [910

University of Sheffield. Courses in Applied Mechanics, 1961–62. Pp. 8. (Sheffield: The University, 1961.) [910

Ciba (A.R.L.), Limited. Technical Notes No. 225: Bonding and Sandwich Construction. By E. J. Catchpole. Pp. 10. (Duxford: Ciba (A.R.L.), Ltd., 1961.) [910

College of Advanced Technology, Birmingham. Annual Report of the Principal, Session 1959–60. Pp. 35. (Birmingham: College of Advanced Technology, 1961.) [910

International Journal of Machine Tool Design and Research, Vol. 1, Nos. 1/2, (September 1961). Pp. 172. Annual subscription rates: (A) For libraries, government establishments, research laboratories, etc. 10 guineas (30 dollars); (B) For individuals who place their order directly with the publisher and certify that the journal is for their personal use 3 guineas (10 dollars). (London and New York: Pergamon Press, 1961.) [910

Scottish Society of the History of Medicine. Report of Proceedings, Session 1960–61. Pp. 36+2 plates. (Edinburgh: Scottish Society of the History of Medicine, 1961.) [910

Short Guide to the Border Forest Park. Pp. 8. (London: H.M. Stationery Office, 1961.) 6d. net. [910

Forestry Commission. Report on Forest Research for the year ended March 1960. Pp. viii+203. (London: H.M. Stationery Office, 1961.) 12s. net. [910

General Register Office. The Registrar General's quarterly return for England and Wales—Births, Deaths and Marriages; Infectious Diseases; Weather; Population Estimates, quarter ended 30th June, 1961, (2nd quarter 1961). Pp. 23. (London: H.M. Stationery Office, 1961.) 2s. 6d. net. [910

Third Report from the Committee of Public Accounts, Session 1960–61. Pp. 40. (London: H.M. Stationery Office, 1961.) 2s. net. [1110

Kodak Limited. Kodak Wratten Filters and other filters manufactured by Kodak Limited, London. Second edition. Pp. 90. (London: Kodak Limited, 1961.) [1110

Memoirs of the British Astronomical Association: Radio-Electronics Section. Vol. 39, No. 2, (August 1961): Artificial Earth Satellites. Compiled and edited by J. Heywood. Pp. viii+160. (Hounslow West, Mddx.: British Astronomical Association, 1961.) 20s.; 3 dollars. [1110

Other Countries

Institutt for Atomenergi, Kjeller, near Lillestrøm, Norway—Descriptive Brochure. Pp. 20. (Kjeller, near Lillestrøm: Institutt for Atomenergi, 1961.) [410

National Science Foundation, Washington, D.C. Surveys of Science Resources Series, NSF 61–32: Funds for Research and Development in Industry, 1958. Pp. xvi+119. (Washington, D.C.: Government Printing Office, 1961.) 65 cents. [410

Die Geschlechtshormone Bildenden Eierstockgeschwülste: Klinische und Pathologische Beobachtungen. Von Antal Jakobovits. (*Studia Medicina Szegedinensia*, Tomus 1, Fasciculus 1.) Pp. 82. (Szeged: The University, 1961.) [410

Commonwealth of Australia. Thirteenth Annual Report of the Commonwealth Scientific and Industrial Research Organization for the year ending June 30, 1961. Pp. iii+150. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1961.) [410

Comptes Rendus des Travaux du Laboratoire Carlsberg. Vol. 32, No. 10: Quantitative Evaluation of the Thiocholine Method for Cholinesterase as Applied to Single End-Plates from Mouse Gastrocnemius Muscle. By Miro Brazil and Erik Zeuthen. Pp. 139–153. 2 kr. 25 øre. Vol. 32, No. 11–12: Cytoplasmic Crystals of the Amoebae, *Amoeba proteus* and *Chaos chaos*. 1: Isolation and Purification of the Crystals and Determination of their Density. By Richard S. Thomas. Cytoplasmic Crystals of the Amoebae, *Amoeba proteus* and *Chaos chaos*. 2: Preparation of Amoeba Crystals in Milligram Quantities. By W. B. Grunbaum. Pp. 155–183+2 plates 4 kr. 75 øre. Vol. 32, No. 13: Statistical Analysis of Enzymic Steady-State Rate Data. By Gordon Johansen and Rufus Lumry. Pp. 185–214. 3kr. 75 øre. (Copenhague: Danish Science Press, Ltd., 1961.) [410

Commonwealth of Australia. Commonwealth Scientific and Industrial Research Organization: Dairy Research Section. Annual Report for the year ending June 30, 1961. Pp. 18. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1961.) [410

National Academy of Sciences—National Research Council. Nuclear Science Series, NAS-NS 3037: The Radiochemistry of Polonium. By P. E. Figgins. Pp. v+68. (Washington, D.C.: Office of Technical Services, Department of Commerce, 1961.) 75 cents. [410

Annals of the New York Academy of Sciences. Vol. 94, Article 2: Current Problems in Electrobiology. By Dominick P. Purpura and 11 other authors. Pp. 337–654. (New York: New York Academy of Sciences, 1961.) [410

Annual Report of the National Museum, Bloemfontein, for the year ended 31st March, 1961. Pp. 16 (Bloemfontein: National Museum, 1961.) [410

Metropolitan Life Insurance Company. Statistical Bulletin, Vol. 42, (August, 1961): Health of the School-Age Population. Accident Hazards at the School Ages. Longevity at All-Time High. Health Trends in Canada. Pp. 12. (New York: Metropolitan Life Insurance Company, 1961.) [410

Bulletin of the American Museum of Natural History. Vol. 122, Article 4: A Generic Revision and Phylogenetic Study of the Family Kalotermitidae (Isoptera). By Kumar Krishna. Pp. 303–408. (New York: American Museum of Natural History, 1961.) 1.50 dollars. [410

Forschungsberichte des Landes Nordrein-Westfalen. Nr. 919: Die Haarfarben der Säugetiere. 1: Spektralanalytische Untersuchungen an Haut-, Haar- und Federmelaninen (Literaturübersicht). Von Dr. Ernst Lubnow und Lisa Kramer. Pp. 75. (Köln und Opladen: Westdeutscher Verlag, 1961.) 21.30 D.M. [410

Western Australia. Report of the Geological Survey Branch for the year 1959. Pp. 50+27 plates. (Perth: Government Printer, 1961.) [410

Centro de Investigaciones Pesqueras, Habana. Departamento de Pesca del Instituto Nacional de Reforma Agraria. Contribución No. 12: Segunda Pesca Exploratoria y Datos Biologicos de la Langosta *Panulirus argus* en Cuba. Por Rene J. Buesa Mas. Pp. 66. (Banta, Habana: Centro de Investigaciones Pesqueras, 1961.) [910

Bulletin of the American Museum of Natural History. Vol. 122, Article 5: Pseudoscorpions from Colorado. By C. Clayton Hoff. Pp. 409–464. (New York: American Museum of Natural History, 1961.) 1 dollar. [910

United States Department of the Interior: Geological Survey, Water-Supply Paper 1531: Hydrology of the Upper Cheyenne River Basin. A: Hydrology of Stock-Water Reservoirs in Upper Cheyenne River Basin. By R. C. Culler. B: Sediment Sources and Drainage-Basin Characteristics in Upper Cheyenne River. By R. F. Hadley and S. A. Schumm. Pp. v+198+2 plates. Water-Supply Paper 1545–A: Microtime Measurement in Aquifer Tests on Open-Hole Artesian Wells. By Granville G. Wyrick and Edwin O. Floyd. Pp. iii+11. 15 cents. Water-Supply Paper 1589: Geology and the Availability of Water in the Lower Bonita Creek Area, Graham County, Arizona. By L. A. Heindl and R. A. McCullough. Pp. iv+56+2 plates. Bulletin 1028–R: Geological Reconnaissance of Kiska Island, Aleutian Islands, Alaska. By Robert R. Coats, Willis H. Nelson, Richard Q. Lewis and Howard A. Powers. Pp. vi+563–581+plates 71–74. (Washington, D.C.: Government Printing Office, 1961.) [910

Eastern Nigeria: Forest Division. Official Document No. 17 (1961): Annual Report, 1960–61. Pp. 26. (Enugu, Eastern Nigeria: Government Printer, 1961.) [910

Berichte des Deutschen Wetterdienstes. Nr. 75, (Band 10): Bestimmung der alten Temperaturwerte von Tübingen und Ulm mit Hilfe von Häufigkeitsverteilungen, (Anhang: Die Beobachtungen von Camerarius, Tübingen (1691–1694) und Algöwer, Ulm (1710–1714)). Von Walter Lenke. Pp. 87. (Offenbach a.M.: Selbstverlag des Deutschen Wetterdienstes, 1961.) [910

Colony of Mauritius. Meteorological Observations and Climatological Summaries. January, 1960. Pp. ii+19. February, 1960. Pp. 19. March, 1960. Pp. 19. April, 1960. Pp. 19. May, 1960. Pp. 19. June, 1960. Pp. 19. (Port Louis: Government Printer, 1961.) [910

Transactions of the American Philosophical Society, New Series, Vol. 51, Part 5: The Juristic Basis of Dynastic Right to the French Throne. By Ralph E. Giesey. Pp. 47. (Philadelphia: American Philosophical Society, 1961.) 1.50 dollars. [910

Krsi-parásara. Edited and translated by Girija Prasanna Majumdar and Sures Chandra Banerji. (*Bibliotheca Indica*: a Collection of Oriental Works. Work No. 285, Issue No. 1579.) Pp. xviii+88+ix+9. (Calcutta: The Asiatic Society, 1960.) Rs. 7.50n.P. [910

Malayan Nature Society. Nature Conservation in Western Malaysia, 1961. Edited by J. Wyatt-Smith and P. R. Wycherley. (*The Malayan Nature Journal*, 21st Anniversary, Special Issue.) Pp. viii+261+44 plates. (Kuala Lumpur: The Malayan Nature Society, 1961.) Soft-back 12s. 6d.; Hard-back 17s. 6d. [910

Ministère de l'Agriculture, Québec. Service de l'Information des Recherches. Publication Nr. 256: Manuel d'Emploi des Herbicides, 1960–1961. Pp. 80. (Québec: Ministère de l'Agriculture, 1961.) [910

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office